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TITLE

Engineering of Microbial Cell Factories for the Production of Plant Polyphenols with Health-Beneficial Properties

RUNNING TITLE

Microbial production of polyphenols

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Abstract

Polyphenols form a group of important natural bioactive compounds with numerous ascribed health-beneficial attributes (*e.g.* antioxidant, anti-inflammatory, anti-microbial and tumor-suppressing properties). Some polyphenols can also be used as natural dyes or plastic precursors. Notwithstanding their relevance, production of most of these compounds still relies on extraction from plant material, which for most of it is a costly and an inefficient procedure. The use of microbial cell factories for this purpose is an emerging alternative that could allow a more efficient and sustainable production. The most recent advances in molecular biology and genetic engineering, combined with the ever-growing understanding of microbial physiology have led to multiple success stories. Production of multiple polyphenolic compounds or their direct precursors has been achieved not only in the common production hosts, such as *Escherichia coli* and *Saccharomyces cerevisiae*, but also in *Corynebacterium glutamicum* and *Lactococcus lactis*. However, boosting production of native compounds or introduction of heterologous biosynthetic pathways also brings certain challenges, such as the need to express, balance and maintain efficient precursor supply. This review will discuss the most recent advances in the field of metabolic engineering of microorganisms for polyphenol biosynthesis and its future perspectives, as well as outlines their potential health benefits and current production methods.

Introduction

There exist over 200,000 different secondary metabolites in plants [1, 2]. Of those, polyphenols are among the most widespread and ubiquitous classes. It has been estimated that in some cases up to 20% of the fixed carbon goes into the phenylpropanoid pathway that leads to the production of the majority of naturally-occurring phenolic compounds [3]. Although polyphenols are classified as secondary metabolites, i.e. molecules that in plants play little or no role in primary metabolism and therefore are not essential for cell's survival under normal conditions, these compounds may accumulate in considerably high amounts [4]. Polyphenols perform many diverse functions in plants, including anti-microbial and anti-fungal protection, insect feeding deterrence, providing coloration to leaves, flowers, and fruits, attraction of pollinators, chelation of toxic heavy metals, protection from UV radiation-induced damage, and free radical scavenging [5–8].

In plants, the aromatic amino acids *L*-phenylalanine and *L*-tyrosine are the two biosynthetic precursors of phenylpropanoid compounds (**Fig. (1)**), [9]). This group consists of compounds with the C₆-C₃ backbone, such as cinnamic acid derivatives, coumarins, and lignans [10]. This backbone can be further extended with up to three two-carbon units derived from malonyl-CoA generating various polyphenols, such as curcuminoids, flavonoids, stilbenes, and styrylpyrones. Of those, the flavonoids (C₆-C₃-C₆ backbone) are the largest group. The vast chemical diversity of flavonoids arises from differences in the backbone structure, as well as from a variety of modifications to the backbone. Possible modifications are acetylation, aryl-migrations, glycosylations, hydroxylations, methylations, polymerizations, and prenylations. Based on these variations, the flavonoids can be further subdivided into aurones, flavanones, flavones, isoflavones, flavonols, flavan-3-ols, anthocyanidins, and tannins (**Fig. (1)**), [10]). A good example of the diversity of the decorations is provided by the anthocyanins, most of which are anthocyanidins glycosylated at the position 3 of the C-ring. Anthocyanins may have additional functional groups such as glycosyl groups (*e.g.* 5-glycosylation and 3'-glycosylation), methyl groups (*e.g.* 3'-methylated petunidin and 7, 3'-methylated rosinidin), hydroxyl groups (which distinguish pelargonidin, cyanidin, and delphinidin derivatives), and acyl groups (on glycosyl moieties, could be both aromatic and/or aliphatic). These decorations can profoundly affect chemical properties such as color, hydrophobicity, and stability, as well as have a strong impact on the compounds bioavailability and bioactivity [11–14].

Polyphenols exhibit an immense natural chemical diversity and appear to have a number of different molecular targets, participating in several signaling pathways, and exhibiting pleiotropic activities both in plants and inside the human body when taken up as a part of diet [13, 15–20]. In addition, their occurrence in plants as complex mixtures makes it possible to take advantage of additive or synergistic activities of such combinations [21–23]. As a result of their diversity in structure and the ethnic knowledge of the use of particular plants in traditional medicine [24, 25], polyphenols have been the subject of intense research with respect to their health benefits [26–28]. Along with their use as pharmaceuticals, polyphenols have found many other potential applications such as natural pigments and food colorants, preservatives, monomers for bioplastics and composites, etc. [29–32].

In recent years the global market for polyphenols has seen continuous growth, with the main booster being the accumulated evidence of polyphenols' health-promoting traits leading to increased sales of polyphenols-containing food supplements, cosmetics, and other type of pharma- and nutraceutical products [33, 34]. In order to keep up with the increasing demand, there is a need for innovative solutions to the large-scale production of such compounds that can replace the less economic and eco-friendly traditional production methods, such as direct purification from plant raw materials or chemical synthesis. In this scenario, bio-based production of chemicals through metabolic engineering of microorganisms has emerged as a viable, affordable, and sustainable alternative. The advent of functional “Omics”, genome-scale modeling, and high-throughput screening technologies, combined with the ever-growing genome engineering, editing, and (heterologous) gene expression toolbox, has brought metabolic engineering to a more systematic and global level, thus significantly reducing the costs associated with the complete development of a novel bioprocess. The variety and complexity of chemicals that can now be produced using microbial cell factories has remarkably increased allowing now even the production of complex polyphenols with multiple biosynthetic pathways steps [35, 36].

This review will first focus on health benefits of different polyphenol groups and their applications. The current extraction and production approaches will then be discussed. Lastly, the ongoing research towards development of alternative production methods with a special emphasis on harnessing the potential of microbes as cell factories for the biosynthesis of polyphenols will be covered.

Polyphenols, their health-beneficial effects, and potential applications

Although primarily known as anti-oxidants, polyphenols possess multiple other health-beneficial properties. Most notably, there are several studies that recommend daily intake of high levels of polyphenols as a mean of reducing risks of cardiovascular disorders and type II diabetes [37–39]. Furthermore, these compounds were demonstrated to have an effect on certain types of cancer, neurodegenerative diseases, allergies, inflammation, and also to alleviate menopausal symptoms by estrogen mimicking [16, 40–44]. Additionally, polyphenols were documented to have anti-inflammatory, anti-aging, anti-angiogenic properties, as well as anti-viral and antimicrobial activities against human pathogens [45–50]. An overview of the specific health-promoting properties of the major groups of polyphenols is given in Table 1.

Bioactivities of polyphenols extend beyond the field of pharmaceuticals, and these compounds are also known for their many applications in food and cosmetics industries as natural substituents for synthetic ingredients. The concern over the safety of synthetic colorants raised by consumer groups [51, 52], combined with the health benefits provided by the many naturally-occurring pigments, has triggered the current marketing trend for replacement of artificial food dyes with natural colors [53]. In this respect, the bright and diverse colors of anthocyanins (hue range from red to bluish-red to purple), together with their health-beneficial properties, relative stability, and high solubility in water, make these flavonoids excellent candidates for food applications (Table 1) [12, 54–56]. As multiple anthocyanins are approved by the European Food Safety Authority [57], a great demand for their use as potential substitutes for the banned synthetic dyes is expected [58–60]. Similarly to colorants, the search for natural solutions to replace currently-used preservatives is also ongoing. Processed foods with minimal synthetic additives and thermal treatment are becoming an increasing trend. Thus, innovative solutions to extend product shelf-life, such as the use of natural preservatives, are required [61, 62]. The antimicrobial activity of flavonoids is a general attribute of this group of compounds, with some specific flavonoids also possessing anti-viral and anti-fungal properties [47, 63]. Moreover, being strong antioxidants [8, 64] flavonoids could potentially protect food from some undesirable chemical changes. Altogether, these attributes make flavonoids a very promising new group of natural preservatives suitable for use in food industry. Cosmetics is another area of interest for polyphenol applications. Multiple plant species that are enriched in polyphenols, such as cocoa, grape, olive, and tea, are used in cosmetic

products [65–67]. The potential of the application of coffee polyphenolic extract and of caffeic acid alone as components for skin care formulations has also been studied [68, 69]. Apart from protecting skin from UV radiation and reactive oxygen species, polyphenols have several other beneficial effects, including antiaging (via inhibition of lipoxygenase, cyclooxygenase, and skin re-modeling enzymes elastase and collagenase), depigmenting (via inhibition of tyrosinase), inhibition of inflammatory responses, and anti-microbial activity [66, 70, 71]. These data provide a good foundation for further research directed towards the application of purified polyphenolic compounds (single or as mixtures) in skincare products.

Current approaches/methods for the production of polyphenols

At the moment all purified polyphenolic compounds available on the market are either obtained through extraction from plant sources (*e.g.* fruits, leaves, or roots), or by total or partial chemical synthesis. So far, resveratrol remains the only exception to these traditional production methods that is also produced by microbial synthesis using *Saccharomyces cerevisiae* (Table 2). The extraction procedures impose numerous limitations that hinder the exploitation of polyphenols for pharmaceutical and biotechnological applications at their full potential [72, 73]. Factors such as low natural abundance inside plant tissues, environmental and geographical conditions, seasonal variation, and the need for complex downstream processing could have a significant impact on the extraction yields of polyphenols. Consequently, the extraction procedures from plant sources are generally labor-intensive, costly, and using plant sources may consume large amounts of resources, such as water and land. Furthermore, the resulting preparations tend to contain impurities. One of the best known examples is the laxative emodin that is often co-purified with and contaminates resveratrol extracts from Japanese knotweed (*Polygonum cuspidatum* Siebold & Zucc.) [74]. Furthermore, despite all the progress made in the field of metabolic engineering of plants and plant cell factories for increased production of native secondary metabolites, their application as production hosts for polyphenols is still limited [75–79]. On the other hand, chemical synthesis of polyphenols has limited options for large-scale production due to the high structural complexity of these molecules. Both *de novo* synthesis and synthesis from purified precursors involves the use of hazardous and toxic chemical solvents, as well as extreme reaction conditions, thus limiting their application to specialized small-scale production [80]. Molecular chirality imposes additional challenges to chemical synthesis, as this process is not stereo-specific and yields a mixture of *R*-

and *S*-stereoisomers, whereas only 2*S*-stereoisomers of polyphenols were shown to be bioactive [81]. Consequently, an extra purification step is required for separating the isomers further reducing the final yield. Hence, more modern and environmentally-minded approaches are required in order to meet the growing demands for these phytochemicals.

Microbial production of plant polyphenols: past achievements and ongoing research

The inefficiency of traditional production methods is a major obstacle to broadening the range of applications for added-value polyphenols, and consequently successful commercialization would require implementation of large-scale, cost-effective, and sustainable production processes. In light of that, construction of designer microorganisms serving as biological platforms for the production of phenolic compounds is becoming a promising alternative. Industrial workhorses, such as *Bacillus subtilis*, *Escherichia coli* or *Saccharomyces cerevisiae*, have been used for decades in numerous bioprocesses, including biological production of compounds with applications in pharmaceutical, food, and chemical industries. Microbial production of fine chemicals presents multiple advantages that have been reviewed previously [82–84]. Briefly, as compared to plants and plant cell cultures, the microorganisms that are used for production are usually fast-growing and easy to cultivate, which greatly reduces processes costs and production times. They are also able to grow in diverse media, including industrial and agricultural waste, which makes the bioproduction more sustainable. Moreover, microbial fermentations are readily scalable from laboratory through demonstration to commercial production scales. Also, the ease of genetic manipulation with these organisms and the availability of molecular tools (*e.g.* for expression of heterologous polyphenol pathway genes, for manipulation of homologous polyphenol pathway genes, or for genome editing) facilitates the construction of microbial cell factories tailored for production of nearly any natural (and even non-natural) metabolite imaginable [83]. Furthermore, the use of microbial hosts for the production of polyphenols simplifies the product purification procedure, as their secondary metabolism is generally much simpler and competing pathways can be eliminated or deactivated. Lastly, as opposed to the traditional methods for obtaining natural products, microbial-based production can be a lot more environmentally-friendly, as the use of organic solvents or other harsh chemicals for product purification can be reduced [85, 86]. Production of fine chemicals

using microorganisms also requires considerably less natural resources, such as extensive land and water usage, as well as fertilizers and pesticides, needed to obtain and process large amounts of raw plant material [87, 88].

Over the past years, multiple studies demonstrated the potential of microbial cell factories for the production of diverse classes of plant natural products (reviewed in [73, 83]). Among the polyphenols, various flavonoids [35, 83], stilbenes [89], raspberry ketone, a raspberry flavor molecule [90], caffeic acid, a lignin precursor [91, 92], and curcuminoids [93, 94] have been heterologously produced in microorganisms. The most interesting examples of polyphenol production using microbial cell factories are summarized in Table 3. There is also an example of production titers, productivity, and yield that meet the targets of the large-scale commercialization being achieved. The Swiss company Evolva is manufacturing resveratrol using yeast (*S. cerevisiae*) as the production platform [95]. Up to now their EveResveratrolTM remains the only marketed phenolic compound produced *de novo* by fermentation in metabolically-engineered microorganisms (Table 2). It is also noteworthy that Evolva has either already established, or is about to initiate microbial production of several other phytochemicals, including the flavor and fragrance ingredient vanillin, the spice saffron, and the natural stevia sweeteners (<http://www.evolva.com/products/>). This example clearly demonstrates the feasibility of commercial bio-based production of plant-borne compounds in microbial cell factories.

From early days, the health benefits ascribed to polyphenols have prompted a significant amount of research work towards the elucidation of their biosynthetic pathways, the genes involved, and their regulation. The information gathered over the past years has led to multiple cases of successful genetic and/or metabolic engineering of whole plants or of plant cell cultures for improved biosynthesis of native and non-native polyphenolic compounds (for selected reviews see [33, 75, 77, 96–99]). This section, however, will only focus on the latest developments in the field of polyphenol production by microorganisms, since these are probably the better candidates for a large-scale and sustainable production.

The first steps of the phenylpropanoid biosynthesis pathway lead to the production of *p*-coumaric acid via deamination of the aromatic amino acids *L*-phenylalanine or *L*-tyrosine (**Fig. (1)**). Production of *p*-coumaric acid from *L*-phenylalanine is a two-step process where the amino acid is first converted into *trans*-cinnamic acid by a phenylalanine ammonia-lyase (PAL), which is further hydroxylated by cinnamate 4-hydroxylase (C4H), a

cytochrome P450 enzyme. The successful production of *p*-coumaric acid from *L*-phenylalanine was first demonstrated in *S. cerevisiae* by co-expression of PAL- and C4H-coding genes [100]. However, expression of this pathway in bacteria presents a challenge due to involvement of the P450 enzyme. These proteins are usually membrane-bound, thus functional expression in prokaryotes is difficult due to lack of the endoplasmic reticulum. Also, cytochromes P450 rely on P450 reductase enzymes (CPR) for cofactor regeneration, which are not present in bacteria and therefore need to be co-introduced into production strains [36]. In contrast, conversion of *L*-tyrosine into *p*-coumaric acid occurs in a single step catalyzed by a tyrosine ammonia-lyase (TAL), circumventing the need for C4H activity. A recent study describes several novel highly-specific TAL enzymes that are functional and produce high levels of *p*-coumaric acid in *E. coli*, *Lactococcus lactis*, as well as in *S. cerevisiae* [101]. Alternatively, use of a promiscuous PAL that can also take up *L*-tyrosine as a substrate for the production of the flavanone naringenin has been reported in *E. coli* [102, 103].

Flavonoids are by far the most explored group of polyphenols in terms of heterologous production in microorganisms [104]. *De novo* biosynthesis of complex flavonoids would require efficient production of the flavonoid core molecules, flavanones. These compounds are synthesized by CoA-esterification of cinnamates, such as *p*-coumaric acid and cinnamic acid, by 4-coumaroyl-CoA ligase (4CL), followed by condensation with three malonyl-CoA molecules catalyzed by chalcone synthase (CHS) and subsequent ring closure by chalcone isomerase (CHI). Further chemical modifications of flavanones, such as hydroxylations, methylations, methoxylations, acylations, and glycosylations, give rise to the vast diversity of flavonoid compounds (**Fig. (1)**). Flavanones such as naringenin and pinocembrin were successfully produced in *E. coli* and *S. cerevisiae* by co-expression of different combinations of PAL/TAL, 4CL, CHS, and CHI enzymes (for an overview see [35, 105–107]). Biosynthesis of a more complex flavanone, eriodictyol, has also been engineered in *E. coli* by additional expression of a flavonoid 3'-hydroxylase (F3'H) enzyme [108]. Furthermore, by combining 4CL, CHS, CHI, and chalcone reductase (CHR), liquiritigenin, 7-hydroxyflavanone, and butin were produced from, respectively, *p*-coumaric acid, cinnamic acid, and caffeic acid as precursors in both *E. coli* and *S. cerevisiae* [109]. To further broaden the spectrum of microbially-produced flavonoids, the biosynthesis of flavones [110, 111] and isoflavones [112] from precursors was achieved through the additional introduction of flavone synthase (FNS) or isoflavone synthase (IFS) genes, respectively. In another study, isoflavone genistein was produced directly from *L*-phenylalanine in yeast [107] and from *L*-tyrosine in *E. coli* [113]. Co-expression of the above-mentioned flavanone biosynthetic genes with flavanone

3-hydroxylase (F3H)- and flavonol synthase (FLS)-coding genes yielded the flavonols, kaempferol from *L*-tyrosine and galangin from *L*-phenylalanine [114]. Similarly, production of the flavonol fisetin from *L*-tyrosine has been recently established in *E. coli* by combining the liquiritigenin biosynthesis genes with the genes coding for F3H, FLS, and F3'H [115]. By combining F3H, dihydroflavonol reductase (DFR) and leucocyanidin reductase (LAR), the production of flavan-3-ols (+)-catechin and (+)-afzelechin was achieved from caffeic acid and *p*-coumaric acid, respectively [116]. Lastly, flavanones have also been converted to anthocyanins in a four-step pathway involving F3H, DFR, anthocyanidin synthase (ANS), and anthocyanidin 3-*O*-glucosyltransferase (3GT) [117, 118].

As mentioned above, flavonoids can be further modified by various decorating enzymes. Such modifications not only alter chemical properties and improve stability, but sometimes also grants the compounds novel biological activities [119, 120]. Thus, modified flavonoids might present additional commercial interest. The most common modification of plant flavonoids is glycosylation, often occurring at least at one position [120]. Multiple studies addressed the issues of glucosylation of flavonols [121, 122], flavones [123, 124], flavanones [125], and isoflavones [122, 126, 127]. Addition of other sugar moieties has been also successfully attempted, including rhamnosylation [121, 128, 129], xylosylation [130, 131], and galactosylation [132]. Lastly, biosynthesis of quercetin 3-*O*-*N*-acetylglucosamine has been reported as well [133].

Methylation is another common modification, and there have been several studies aiming at microbial biosynthesis of methylated flavonoids. One such example is the work of Kim *et al*, where *E. coli* strains for the production of ponciretin (4'-*O*-methylnaringenin) and sakuranetin (7-*O*-methylnaringenin) were constructed [134]. Other examples refer to the construction of strains producing the medically-important flavanonol 7-*O*-methyl aromadendrin from *p*-coumaric acid [135] and the flavonol genkwanin (7-*O*-methyl apigenin) from *L*-tyrosine.

Compared to flavonoids, microbial production of stilbenes is somewhat less of a hot topic. Nevertheless, numerous health benefits attributed to this group of polyphenols did stimulate research efforts to produce them heterologously in microorganisms for various applications in pharmaceutical and food industries. Similarly to flavonoids, stilbenes are produced via decarboxylative condensation of three malonyl-CoA molecules with the CoA-activated hydroxycinnamates through the action of stilbene synthase (STS) (**Fig. (1)**). Original attempts to establish microbial production of stilbene resveratrol were mainly done by the co-expression of the *4cl* and the *sts* genes, and have been accomplished in both *S. cerevisiae* and *E. coli* [136–140]. A more systematic approach comprising the use of two

different production strains, two promoter systems, screening of a *sts* gene library, and fine-tuning of gene expression levels further improved the production of resveratrol from *p*-coumaric in *E. coli* [141]. The engineered strain *E. coli* BW27784 expressing the *4cl* gene from *Arabidopsis thaliana* and the *sts* gene from *Vitis vinifera* organized in a bi-cistronic operon on a high-copy number plasmid, accumulated the impressive amount of 2.4 g/L resveratrol after the addition of a fatty acid biosynthesis inhibitor to improve precursor availability. Research efforts to bypass the use of the expensive precursor *p*-coumaric acid by supplying external L-phenylalanine or L-tyrosine resulted in consistently low titers of resveratrol [104, 107, 142, 143]. However, extensive strain optimization through i) increase of the availability of L-phenylalanine and malonyl-CoA, ii) integration of the resveratrol biosynthetic pathway in the genome and iii) introduction of a resveratrol exporter resulted in a *S. cerevisiae* strain capable of producing 4 g/L of resveratrol from glucose in a fed-batch fermentation [95]. More recently, resveratrol was also produced through *de novo* biosynthesis from both glucose and ethanol via the L-tyrosine intermediate at approximately 0.5g/L [144]. Biosynthesis of pinosylvin from L-phenylalanine has been also reported [145, 146]. Several other studies focused on the production of methylated resveratrol derivatives, such as the mono-methylated pinostilbene and the di-methylated pterostilbene, which are equally or sometimes even considerably more bioactive than resveratrol [147]. Production of both pinostilbene and pterostilbene from *p*-coumaric acid was achieved by additional expression of stilbene *O*-methyltransferases (OMTs) genes from various sources in both *S. cerevisiae* and *E. coli* [148, 149]. Furthermore, another study has reported production of the unnatural stilbene methyl ethers by the expression of *Oryza sativa* OsOMT1 in *E. coli* [145].

The ever-increasing knowledge of specific pathways and the discovery of novel enzymes have contributed to the microbial production of difficult-to-synthesize polyphenols, such as certain phenolic acids or coumarins, whose biosynthesis involves production of cytochrome P450 enzymes or *O*-methyl-transferases. Identification of several bacteria-compatible hydroxylases that can replace *p*-coumarate 3-hydroxylase (C3H, a P450 enzyme) has made it possible to engineer artificial pathways for the biosynthesis of caffeic and ferulic acids from L-tyrosine or *p*-coumaric acid [91, 142, 150–152]. Maximal concentrations of 767 mg/L of caffeic acid and 196 mg/L ferulic acid were produced *de novo* by L-tyrosine overproducer strains of *E. coli* expressing, respectively, TAL and 4-hydroxyphenylacetate 3-hydroxylase [150] or TAL, C3H, and a caffeic acid methyltransferase (COM) [152]. Production of plant-specific coumarins in bacteria has been also described [153]. At the first stage, *E. coli* strains were engineered to convert the phenylpropanoid acid precursors, *p*-coumaric acid and ferulic acid, into the simple

coumarins, umbelliferone (4.3 mg/L) and scopoletin (27.8 mg/L), respectively. Furthermore, these two coumarins were later-on produced *de novo* without the addition of any precursor after assembling the complete artificial biosynthetic pathway in *E. coli*. This pioneering study set the foundation for microbial production of more diverse coumarin molecules. Coumarins are components of various polymers [154] and were also demonstrated to have analgesic and anti-inflammatory properties [155], thus their production in microbial cell factories could also be of a commercial interest.

Although the use of *S. cerevisiae* and *E. coli* presents multiple advantages, other microorganisms might be more suitable for production of polyphenolic compounds, for example due to higher end-product tolerance or a broader range of growth substrates. One of the first attempts of using a non-conventional host for the flavonoid production was done in *Streptomyces venezuelae*, where the flavanones naringenin and pinocembrin, as well as the stilbenes resveratrol and pinosylvin were produced from, *p*-coumaric acid and cinnamic acid, respectively [156]. The same organism was later on engineered for the biosynthesis of kaempferol and galangin by the co-expression of the *f3h* gene from *Citrus siensis* and the *fls* gene from *Citrus unshius* and feeding with naringenin or pinocembrin, respectively [157]. A more recent study has demonstrated the feasibility of polyphenol production in *Corynebacterium glutamicum*, a soil bacterium that is used for amino acid production on industrial scale. Kallscheuer *et al*, were able to engineer this bacterium to produce resveratrol and naringenin directly from glucose with yields comparable to those observed in *E. coli* [158]. Moreover, *C. glutamicum* was further engineered for the production of resveratrol from 4-hydroxybenzoic acid (HBA) by reversal of a β -oxidative phenylpropanoid degradation pathway [159]. This allows having polyphenol production independent from the aromatic amino acid biosynthesis. The most “exotic” case was a study where the edible macrofungus *Tremella fuciformis* Berk. (silver ear or white jelly mushroom, division Basidiomycota) was genetically modified for the biosynthesis of resveratrol from *p*-coumaric acid with yields of 0.8-0.9 μ g/g of dry weight after 7 days of cultivation [160].

Strategies for improving production of polyphenolic compounds in microorganisms

Metabolic engineering is the introduction of targeted adjustments to cellular metabolic processes aiming at improving production of a certain substance. This is often achieved with a set of genetic manipulations that leads to alterations within various regulatory, enzymatic, and transport functions of the cell [161, 162]. Metabolic engineering is particularly important for optimization of heterologous pathways, as introduction of such pathways

often leads to flux imbalance, not only within the pathway, but also often within the global cellular metabolism. This occurs because the host generally lacks the regulatory machinery required for efficient and balanced operation of the pathway, and also to prevent over- or under-production of enzymes, leading to metabolic burden on the host at the cost of productivity of the compound of interest, as well as accumulation of potentially toxic intermediates [163].

One of the key targets for metabolic pathway engineering is the improvement of precursor supply. The most notable strategies for that are summarized in Table 4. Numerous studies dealing with polyphenol production have concluded that increasing the intracellular pool of malonyl-CoA is the key requirement for enhancing the flavonoid and stilbene production [112, 164, 165]. The most common strategies for that are a) the overexpression of an acetyl-CoA carboxylase (ACC)-coding gene that converts acetyl-CoA into malonyl-CoA and b) the inhibition of fatty acid biosynthesis by addition of the antibiotic cerulenin [35, 146, 166, 167]. Further improvement could be achieved through the fine-tuning of the acetate assimilation via the overexpression of acetyl-CoA synthetase gene and the deletion of the acetate-utilizing pathways, which overall resulted in a 16.3-fold increase of intracellular malonyl-CoA concentration [108, 166]. Other alternatives include the introduction of a malonate catabolic pathway [168, 169], the overexpression of the 3-ketoacyl-ACP synthase genes *fabH* and *fabF* [166, 170], and the conditional down-regulation of fatty acid biosynthesis with CRISPR interference (CRISPRi) [171]. Several other studies in *E. coli* have taken a more global approach, combining computational predictions and experimental validations [165, 172, 173]. The utilized genome-scale models predicted a set of genetic interventions, mainly aiming to up-regulate some of the glycolytic reactions and down-regulate the tricarboxylic acid (TCA) cycle that cooperatively drive the carbon flux towards malonyl-CoA, while at the same time preventing the formation of byproducts. These interventions were experimentally validated using a lab-scale fermenter, and the introduced genetic modifications resulted in a significantly improved production of naringenin (474 mg/L, [172]) and resveratrol (1600 mg/L, [173]).

Other substrates/co-factors critical for the biosynthesis of flavonoids are the UDP-glucose and NADPH [117, 169, 174]. The former one is the donor of the glucosyl group in the anthocyanin biosynthesis as well as in other flavonoid biosynthetic routes, whereas the latter one is required for the biosynthesis of leucoanthocyanidins, 5-deoxyflavanones, and (+)-catechins (**Fig. (1)**). Engineering of UDP-glucose levels through a combination of overexpressing genes of the UTP biosynthetic pathway, supplementation with orotic acid, and deletion of several endogenous UDP-glucose-utilizing genes resulted in the significantly improved anthocyanin production from

flavan-3-ols precursors in an *E. coli* strain expressing ANS- and 3GT-coding genes [169]. Recently, a follow-up study has reported that overexpression of the *ycjU* gene, which catalyzes the conversion of β anomer of glucose-6-phosphate into glucose-1-phosphate, further increases the UDP-glucose pools, and consequently anthocyanin production [118]. With regard to improving the intracellular NADPH availability, a stoichiometry-based model was deployed to identify a set of potential gene knock-out combinations in *E. coli*. Upon validation of the candidates, the combined inactivation of phosphoglucose isomerase, phosphoenolpyruvate carboxylase, and phospholipase activities resulted in a 4-fold increase of leucoanthocyanidin production and a 2-fold increase of (+)-catechin production, as compared to the wild-type background [174].

Another challenge to the current metabolic engineering strategies is the use of media supplemented with expensive precursors (*e.g.* *p*-coumaric acid or naringenin) and, a two step-fermentation process (biomass/protein production and polyphenol synthesis) that becomes a disadvantage when the process scale-up is considered. Partly, this issue comes from the fact that biosynthetic pathways for complex polyphenols such as flavonols and anthocyanins consist of six or more genes. Overexpression of such high number of genes would first of all cause a large metabolic burden to the cell. This problem has already been partly addressed by using a co-culture strategy [175]. With this approach, Jones *et al.* have split the (+)-afzelechin biosynthetic pathway between the two co-incubated strains: the first one was expressing the malonyl-CoA-dependent part (from *p*-coumaric acid to naringenin), whereas the second strain was expressing the NADPH-dependent downstream part (from naringenin to (+)-afzelechin). Another advantage of the co-cultivation system is that the two strains could be independently engineered for enhanced precursor supply (*e.g.* malonyl-CoA or NADPH) without significantly impacting the cellular metabolism. One of the major parameters that need to be considered and optimized in such experiments is strain compatibility. The selected strains must have similar growth kinetics to avoid out competition of one, which can lead to an imbalance in the pathway. The authors addressed this issue by introducing the plasmids containing the biosynthetic genes into multiple background strains and selecting the most fitting combination. Equal growth could also potentially be ensured by introducing two different auxotrophies in the production strains in a way that would make them dependent on one another. Furthermore, production of compounds that are toxic to other members of the consortium must be avoided and efficient transfer of pathway metabolites from one partner to another must be ensured. The second issue is that expression of this many genes could be challenging due to lack of compatible sets of overexpression vectors for many industrially-relevant microorganisms. This, however, is not an issue for *E. coli* where the complete

370 biosynthetic pathway for the flavonol fisetin from *L*-tyrosine consisting of seven genes has been established using
371 the DUET vector system, in which genes were expressed in pairs utilizing four different expression vectors [115].
372 There is also an interesting solution in *S. cerevisiae* that involves the use of a polyprotein system allowing co-
373 transcription of multiple coding sequences from a single promoter. The system has already been used for the
374 production of 2-hydroxynaringenin-*C*-glucoside [176]. Furthermore, in order to allow complete *de novo*
375 biosynthesis, both bacterial and yeast strains have been engineered for the production of flavonoids and stilbenes
376 from inexpensive substrates, such as glucose, by introducing heterologous genes coding for various polyphenol
377 biosynthesis pathways into *L*-tyrosine- or *L*-phenylalanine-overproducing strains [168, 177–179].

378 Microbial production of polyphenols is often challenged by toxicity of the end-product and/or of its biosynthetic
379 intermediates, as well as by the formation of inclusion bodies resulting from protein overproduction. The former
380 issue is related to anti-microbial properties of polyphenols, which could become an issue particularly if the produced
381 molecules accumulate intracellularly at high concentrations. One possible approach to resolve this is to co-express
382 an exporter protein that would extrude the produced polyphenols into the culture medium [95]. Another approach is
383 to use adaptive laboratory evolution (ALE), which consists of continuous cultivation of the producer microorganism
384 while subjecting it to increasing concentrations of the polyphenols. This process generally results in accumulation of
385 mutations that would increase tolerance of the producer strain towards the target compound [180, 181]. Furthermore,
386 cytotoxic effect of the biosynthetic intermediates could be avoided by balancing expression levels of individual
387 genes within the given pathway [168, 182]. The second issue arises from the necessity for some enzymes to form
388 complexes in order to ensure high local substrate concentrations, in particular if a reaction is unfavorable [82].
389 However, this could also be of an advantage even if the coupling is unnatural, as it would ensure efficient flux from
390 one step of a pathway to another. There are multiple ways of ensure close proximity of biosynthetic enzymes and
391 their intermediates, including intracellular compartmentalization [82], use of synthetic scaffolds [183], and
392 construction of translational fusions. The latter approach has been used multiple times for engineering of polyphenol
393 production, including construction of the 4CL::STS fusion for enhancing resveratrol production [137], construction
394 of CHS::CHR fusion for increasing liquiritigenin production [115], and use of P450::CPR to allow functional
395 expression of P450 enzymes in bacteria [108, 112, 115].

A recent report has drawn the attention to the interference of native aromatic acid degradation pathways with the production of polyphenols [135]. Detailed analysis of *E. coli* strains producing 7-*O*-methyl aromadendrin showed that the final concentration of this flavanone did not correspond to the consumption of *p*-coumaric acid, indicating possible degradation of the precursor via an unknown pathway [184]. *S. cerevisiae* has been also reported to have more than one enzyme catalyzing decarboxylation of *trans*-cinnamic acid and *p*-coumaric acid, a reaction which could also potentially reduce production of polyphenols [185]. A similar situation was observed in *C. glutamicum* where a phenylpropanoid degradation gene cluster had to be deleted prior to engineering of this bacterium for stilbene and flavonoid production [158, 186]. Therefore, possible presence of such enzymes and pathways needs to be accounted for, in particular prior to exploration of a new production host.

Another interesting recent development is the emergence of combinatorial gene expression techniques that appear to be promising approaches to address the challenge of improving titers and productivity efficiency [163, 187]. However, their successful application is highly dependent on the availability of high-throughput methods for strain screening [188]. Recently, two flavonoid biosensors were constructed consisting of the reporter gene coding for the fluorescent protein placed under control of the flavonoid-responsive transcriptional regulator [189]. The transcriptional regulators FdeR from *Herbaspirillum seropedicae* SmR1 was used to generate a biosensor to detect naringenin, whereas QdoR from *B. subtilis* was used to detect quercetin and kaempferol. The QdoR-based biosensor was highly efficient in detecting kaempferol production *in vivo* at the single cell level while using fluorescence-activated cell sorting (FACS). The developed biosensors could be subsequently used for identification of novel genes involved in polyphenol biosynthetic pathways [189]. Another biosensor has been developed based on the *B. subtilis* transcription factor FapR that is responsive to malonyl-CoA [190]. This sensor could therefore be used for selection of candidates with increased intracellular concentrations of malonyl-CoA. Liu *et al.* [191] have used the same transcription factor in order to develop a negative feedback regulatory circuit. The circuit relies on a malonyl-CoA-based sensor-actuator system that controls expression of the *acc* gene, and in this way alleviates the toxic effects of high intracellular concentration of the enzyme. The circuit was proven to be efficient in regulating the fatty acid biosynthetic pathway and increasing fatty acid titer and productivity [191]. Application of such system for microbial production of polyphenols should allow balancing the engineered pathways and subsequently improve the production efficiency. Other approaches and techniques that have been successfully utilized for fine-tuning gene expression for the needs of metabolic engineering were thoroughly reviewed in [192]. Furthermore, a new screening

technique based on high-performance thin-layer chromatography (HPTLC) has been developed for the discovery of flavonoid-modifying enzymes [193]. The authors claim that this metagenome extract thin-layer chromatography analysis (META) allows rapid detection of glycosyltransferases and other flavonoid-decorating enzymes, as well as that the system is highly sensitive, being able to detect of as little as 4 ng of a modified molecule.

Conclusion

There has been a substantial progress in the field of microbial production of polyphenols. The recent advances in genome editing, combined with novel engineering tools, now allow the expression of multiple genes coding for enzymes forming complex biosynthetic polyphenol pathways not only in model organisms such *E. coli* and *S. cerevisiae*, but also in more novel productions hosts, such as *C. glutamicum*, *L. lactis*, and *Streptomyces venezuelae*. Nonetheless, in many cases the production efficiency using microbial hosts remains inferior as compared to extraction from plants. However, constant advances of synthetic biology tools combined with future metabolic engineering efforts will further facilitate the development of more economically-favorable production processes.

Abbreviations

3GT – anthocyanidin 3-*O*-glycosyltransferase, 4CL – 4-coumaroyl-CoA ligase, AAT – anthocyanin acyltransferase, ACC – acetyl-CoA carboxylase, ALE – adaptive laboratory evolution, AMT – anthocyanin methyltransferase, ANR – anthocyanidin reductase, ANS – anthocyanidin synthase (leucoanthocyanidin dioxygenase), C3H – *p*-coumarate 3-hydroxylase, C4H – cinnamate 4-hydroxylase, CDW – cell dry weight, CHI – chalcone isomerase, CHR – chalcone reductase, CHS – chalcone synthase, CoA – Coenzyme A, COM – caffeic acid methyltransferase, CPR – cytochrome P450 reductase, CRISPRi – clustered regularly-interspaced short palindromic repeats interference, CUS – curcuminoid synthase, CVD – cardiovascular diseases, DFR – dihydroflavonol 4-reductase, F3'H – flavonoid 3'-hydroxylase, F3H – flavanone 3-hydroxylase, FACS – fluorescence-activated cell sorting, FLS – flavonol synthase, FNS – flavone synthase, HBA – 4-hydroxybenzoic acid, HTC – high-throughput screening, IFS – isoflavone synthase, K/O – knock-out, LAR – leucoanthocyanidin reductase, NADPH – nicotinamide adenine dinucleotide phosphate, O/E – overexpression, OMT – 3-*O*-methyltransferase, LDL – low-density lipoprotein, PAL – phenylalanine ammonia-lyase, STS – stilbene synthase, TAL – tyrosine ammonia-lyase, TCA cycle – tricarboxylic acid cycle, UDP-glucose – uridine diphosphate glucose.

450

451 **Conflict of Interest**

452 The authors declare no conflicts of interest.

453

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461 **References**

462 [1] Weckwerth W. Metabolomics in systems biology. *Annu Rev Plant Biol* 2003; 54: 669–689.

463 [2] Saito K, Matsuda F. Metabolomics for Functional Genomics, Systems Biology, and Biotechnology. *Annu*
464 *Rev Plant Biol* 2010; 61: 463–489.

465 [3] Pereira DM, Valentão P, Pereira JA, et al. Phenolics: From Chemistry to Biology. *Molecules* 2009; 14:
466 2202–2211.

467 [4] Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health.
468 *Nat Prod Rep* 2009; 26: 1001–1043.

469 [5] Parr AJ, Bolwell GP. Phenols in the plant and in man. The potential for possible nutritional enhancement of
470 the diet by modifying the phenols content or profile. *J Sci Food Agric* 2000; 80: 985–1012.

- 471 [6] Sutherland ORW, Russell GB, Biggs DR, et al. Insect feeding deterrent activity of phytoalexin
472 isoflavonoids. *Biochem Syst Ecol* 1980; 8: 73–75.
- 473 [7] Michalak A. Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal
474 Stress. *Polish J Environ Stud* 2006; 15: 523–530.
- 475 [8] Stevenson DE, Hurst RD. Polyphenolic phytochemicals – just antioxidants or much more? *Cell Mol Life Sci*
476 2007; 64: 2900–2916.
- 477 [9] Iandolino AB, Cook DR. Phenylpropanoid Metabolism in Plants: Biochemistry, Functional Biology, and
478 Metabolic Engineering. In: Fraga CG (ed) *Plant Phenolics and Human Health*. Hoboken, NJ, USA: John
479 Wiley & Sons, Inc., pp. 489–563.
- 480 [10] Springob K, Kutchan TM. Introduction to the different classes of natural compounds. In: Osbourn AE,
481 Lanzotti V (eds) *Plant-derived Natural Products: Synthesis, Function, and Application*. New York, USA:
482 Springer, 2009, pp. 22–26.
- 483 [11] Glover BJ, Martin C. Anthocyanins. *Curr Biol* 2012; 22: R147–50.
- 484 [12] Kong J-M, Chia L-S, Goh N-K, et al. Analysis and biological activities of anthocyanins. *Phytochemistry*
485 2003; 64: 923–933.
- 486 [13] Le Roy J, Huss B, Creach A, et al. Glycosylation Is a Major Regulator of Phenylpropanoid Availability and
487 Biological Activity in Plants. *Front Plant Sci* 2016; 7: 735.
- 488 [14] Tanaka Y, Sasaki N, Ohmiya A. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids.
489 *Plant J* 2008; 54: 733–749.
- 490 [15] Kim J, Lee HJ, Lee KW. Naturally occurring phytochemicals for the prevention of Alzheimer’s disease. *J*
491 *Neurochem* 2010; 112: 1415–1430.
- 492 [16] Ververidis F, Trantas E, Douglas C, et al. Biotechnology of flavonoids and other phenylpropanoid-derived
493 natural products. Part I: Chemical diversity, impacts on plant biology and human health. *Biotechnol J* 2007;

494 2: 1214–1234.

495 [17] Chen D, Milacic V, Chen MS, et al. Tea polyphenols, their biological effects and potential molecular targets.
 496 *Histol Histopathol* 2008; 23: 487–96.

497 [18] Santangelo C, Vari R, Scazzocchio B, et al. Polyphenols, intracellular signalling and inflammation. *Ann*
 498 *dell'Istituto Super di sanità* 2007; 43: 394–405.

499 [19] Kishimoto Y, Tani M, Kondo K. Pleiotropic preventive effects of dietary polyphenols in cardiovascular
 500 diseases. *Eur J Clin Nutr* 2013; 67: 532–535.

501 [20] Kim H-S, Quon MJ, Kim J. New insights into the mechanisms of polyphenols beyond antioxidant
 502 properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. *Redox Biol* 2014; 2: 187–195.

503 [21] Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of
 504 phytochemicals. *Am J Clin Nutr* 2003; 78: 517S–520.

505 [22] Lee KW, Lee HJ, Lee CY. Vitamins, phytochemicals, diets, and their implementation in cancer
 506 chemoprevention. *Crit Rev Food Sci Nutr* 2004; 44: 437–452.

507 [23] Wagner H. Synergy research: Approaching a new generation of phytopharmaceuticals. *Fitoterapia* 2011; 82:
 508 34–37.

509 [24] Tseng and Yean-Jang Lee T-H. Evaluation of Natural and Synthetic Compounds from East Asiatic Folk
 510 Medicinal Plants on the Mediation of Cancer. *Anticancer Agents Med Chem* 2006; 6: 347–365.

511 [25] Gopalan A, Reuben SC, Ahmed S, et al. The health benefits of blackcurrants. *Food Funct* 2012; 3: 795–809.

512 [26] Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. *J*
 513 *Ethnopharmacol* 2000; 71: 23–43.

514 [27] Barnes S, Prasain J. Current progress in the use of traditional medicines and nutraceuticals. *Curr Opin Plant*
 515 *Biol* 2005; 8: 324–328.

- 516 [28] Huang W-Y, Cai Y-Z, Zhang Y. Natural Phenolic Compounds From Medicinal Herbs and Dietary Plants:
517 Potential Use for Cancer Prevention. *Nutr Cancer* 2009; 62: 1–20.
- 518 [29] Bąkowska-Barczak A. Acylated anthocyanins as stable, natural food colorants - a review. *Polish J Food*
519 *Nutr Sci* 2005; 14: 107–116.
- 520 [30] Mo H, Zhu Y, Chen Z. Microbial fermented tea – a potential source of natural food preservatives. *Trends*
521 *Food Sci Technol* 2008; 19: 124–130.
- 522 [31] Li T, Li J, Hu W, et al. Shelf-life extension of crucian carp (*Carassius auratus*) using natural preservatives
523 during chilled storage. *Food Chem* 2012; 135: 140–145.
- 524 [32] Harlin A. Biogenic Precursors for Polyphenol, Polyester and Polyurethane Resins. In: *Handbook of*
525 *Bioplastics and Biocomposites Engineering Applications*. Hoboken, NJ, USA: John Wiley & Sons, Inc., pp.
526 511–553.
- 527 [33] Georgiev V, Ananga A, Tsoleva V. Recent advances and uses of grape flavonoids as nutraceuticals.
528 *Nutrients* 2014; 6: 391–415.
- 529 [34] Ciriminna R, Meneguzzo F, Fidalgo A, et al. Extraction, benefits and valorization of olive polyphenols. *Eur*
530 *J Lipid Sci Technol* 2016; 118: 503–511.
- 531 [35] Pandey RP, Parajuli P, Koffas MAG, et al. Microbial production of natural and non-natural flavonoids:
532 Pathway engineering, directed evolution and systems/synthetic biology. *Biotechnol Adv* 2016; 34: 634–662.
- 533 [36] Dvora H, Koffas MAG. Microbial production of flavonoids and terpenoids. In: McNeil B, Archer D,
534 Giavasis I, et al. (eds) *Microbial Production of Food Ingredients, Enzymes and Nutraceuticals*. Oxford, UK:
535 Woodhead Publishing, pp. 234–261.
- 536 [37] Scalbert A, Manach C, Morand C, et al. Dietary Polyphenols and the Prevention of Diseases. *Crit Rev Food*
537 *Sci Nutr* 2005; 45: 287–306.
- 538 [38] Yamagata K, Tagami M, Yamori Y. Dietary polyphenols regulate endothelial function and prevent

539 cardiovascular disease. *Nutrition* 2015; 31: 28–37.

540 [39] Xiao JB, Hogger P. Dietary Polyphenols and Type 2 Diabetes: Current Insights and Future Perspectives.
541 *Curr Med Chem* 2015; 22: 23–38.

542 [40] Xiao Z-P, Peng Z-Y, Peng M-J, et al. Flavonoids Health Benefits and Their Molecular Mechanism. *Mini-*
543 *Reviews Med Chem* 2011; 11: 169–177.

544 [41] Martin C. The interface between plant metabolic engineering and human health. *Curr Opin Biotechnol* 2013;
545 24: 344–353.

546 [42] Rahman I, Biswas SK, Kirkham PA. Regulation of inflammation and redox signaling by dietary
547 polyphenols. *Biochem Pharmacol* 2006; 72: 1439–1452.

548 [43] Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. *Am J Clin Nutr* 2005; 81:
549 215S–217S.

550 [44] Chirumbolo S. Dietary Assumption of Plant Polyphenols and Prevention of Allergy. *Curr Pharm Des* 2014;
551 20: 811–839.

552 [45] Carrizzo A, Forte M, Damato A, et al. Antioxidant effects of resveratrol in cardiovascular, cerebral and
553 metabolic diseases. *Food Chem Toxicol* 2013; 61: 215–226.

554 [46] Kasiotis KM, Pratsinis H, Kletsas D, et al. Resveratrol and related stilbenes: their anti-aging and anti-
555 angiogenic properties. *Food Chem Toxicol* 2013; 61: 112–120.

556 [47] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. *Sci World J* 2013;
557 2013: 162750.

558 [48] Thapa M, Kim Y, Desper J, et al. Synthesis and antiviral activity of substituted quercetins. *Bioorg Med*
559 *Chem Lett* 2012; 22: 353–356.

560 [49] Russo GL, Russo M, Spagnuolo C, et al. Quercetin: A Pleiotropic Kinase Inhibitor Against Cancer. In:

- 561 Zappia V, Panico S, Russo GL, et al. (eds) *Advances in Nutrition and Cancer*. Berlin: Springer, pp. 185–
562 205.
- 563 [50] Sekizawa H, Ikuta K, Mizuta K, et al. Relationship between polyphenol content and anti-influenza viral
564 effects of berries. *J Sci Food Agric* 2013; 93: 2239–2241.
- 565 [51] Amchova P, Kotolova H, Ruda-Kucerova J. Health safety issues of synthetic food colorants. *Regul Toxicol*
566 *Pharmacol* 2015; 73: 914–922.
- 567 [52] El-Wahab HMFA, Moram GSE-D. Toxic effects of some synthetic food colorants and/or flavor additives on
568 male rats. *Toxicol Ind Health* 2013; 29: 224–32.
- 569 [53] Wrolstad RE, Culver CA. Alternatives to Those Artificial FD&C Food Colorants. *Annu Rev Food Sci*
570 *Technol* 2012; 3: 59–77.
- 571 [54] Melo MJ, Pina F, Andary C. Anthocyanins : Nature’s Glamorous Palette. In: Bechtold T, Mussak R (eds)
572 *Handbook of Natural Colorants*. John Wiley & Sons, Ltd., 2009, pp. 135–150.
- 573 [55] Mercadente AZ, Bobbio FO. Anthocyanins in foods: occurrence and physicochemical properties. In:
574 Socaciu C (ed) *Food Colorants: Chemical and Functional Properties*. CRC Press, 2008, pp. 241–268.
- 575 [56] Riaz M, Zia-Ul-Haq M, Saad B. Biosynthesis and Stability of Anthocyanins. In: Hartel RW (ed)
576 *Anthocyanins and Human Health: Biomolecular and therapeutic aspects*. Springer International Publishing,
577 pp. 71–86.
- 578 [57] EFSA Panel on Food Additives and Nutrient. Scientific Opinion on the re-evaluation of anthocyanins (E
579 163) as a food additive. *EFSA J* 2013; 11: 3145.
- 580 [58] Ignat I, Volf I, Popa VI. A critical review of methods for characterisation of polyphenolic compounds in
581 fruits and vegetables. *Food Chem* 2011; 126: 1821–1835.
- 582 [59] Scotter MJ. Emerging and persistent issues with artificial food colours: natural colour additives as
583 alternatives to synthetic colours in food and drink. *Qual Assur Saf Crop Foods* 2011; 3: 28–39.

- 584 [60] Motohashi N, Sakagami H. Anthocyanins as Functional Food Colors. In: Motohashi N (ed) *Bioactive*
585 *Heterocycles VII: Flavonoids and Anthocyanins in Plants, and Latest Bioactive Heterocycles II*. New York:
586 Springer, pp. 1–40.
- 587 [61] Juneja VK, Dwivedi HP, Yan X. Novel natural food antimicrobials. *Annu Rev Food Sci Technol* 2012; 3:
588 381–403.
- 589 [62] Negi PS. Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food
590 application. *Int J Food Microbiol* 2012; 156: 7–17.
- 591 [63] Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents* 2005; 26: 343–356.
- 592 [64] Einbond LS, Reynertson KA, Luo X-D, et al. Anthocyanin antioxidants from edible fruits. *Food Chem*
593 2004; 84: 23–28.
- 594 [65] Aburjai T, Natsheh FM. Plants used in cosmetics. *Phyther Res* 2003; 17: 987–1000.
- 595 [66] Soto M, Falqué E, Domínguez H. Relevance of Natural Phenolics from Grape and Derivative Products in
596 the Formulation of Cosmetics. *Cosmetics* 2015; 2: 259–276.
- 597 [67] Abdul Wahab N, Rahman RA, Ismail A, et al. Assessment of Antioxidant Capacity, Anti-collagenase and
598 Anti-elastase Assays of Malaysian Unfermented Cocoa Bean for Cosmetic Application. *Nat Prod Chem*
599 *Res*; 2. Epub ahead of print 2014. DOI: 10.4172/2329-6836.1000132.
- 600 [68] Huber P, Adlhart C, Luginbuhl V, et al. Coffee based polyphenols with potential in skin care Antioxidant
601 activity and skin penetration assessed by in vivo Raman spectroscopy. *Househ Pers Care Today* 2014; 9:
602 28–34.
- 603 [69] Magnani C, Isaac VLB, Correa MA, et al. Caffeic acid: a review of its potential use in medications and
604 cosmetics. *Anal Methods* 2014; 6: 3203.
- 605 [70] Ratz-Lyko A, Arct J, Majewski S, et al. Influence of Polyphenols on the Physiological Processes in the Skin.
606 *Phyther Res* 2015; 29: 509–517.

- 607 [71] Zillich O V., Schweiggert-Weisz U, Eisner P, et al. Polyphenols as active ingredients for cosmetic products.
608 *Int J Cosmet Sci* 2015; 37: 455–464.
- 609 [72] Kolewe ME, Gaurav V, Roberts SC. Pharmaceutically Active Natural Product Synthesis and Supply via
610 Plant Cell Culture Technology. *Mol Pharm* 2008; 5: 243–256.
- 611 [73] Wang J, Guleria S, Koffas MA, et al. Microbial production of value-added nutraceuticals. *Curr Opin*
612 *Biotechnol* 2016; 37: 97–104.
- 613 [74] Omar JM, Yang H, Li S, et al. Development of an Improved Reverse-Phase High-Performance Liquid
614 Chromatography Method for the Simultaneous Analyses of *trans* -/ *cis* -Resveratrol, Quercetin, and Emodin
615 in Commercial Resveratrol Supplements. *J Agric Food Chem* 2014; 62: 5812–5817.
- 616 [75] Staniek A, Bouwmeester H, Fraser PD, et al. Natural products - modifying metabolite pathways in plants.
617 *Biotechnol J* 2013; 8: 1159–1171.
- 618 [76] Falcone Ferreyra ML, Rius SP, Casati P. Flavonoids: biosynthesis, biological functions, and
619 biotechnological applications. *Front Plant Sci* 2012; 3: 222.
- 620 [77] Pollier J, Moses T, Goossens A. Combinatorial biosynthesis in plants: a (p)review on its potential and future
621 exploitation. *Nat Prod Rep* 2011; 28: 1897–1916.
- 622 [78] Wu S, Chappell J. Metabolic engineering of natural products in plants; tools of the trade and challenges for
623 the future. *Curr Opin Biotechnol* 2008; 19: 145–152.
- 624 [79] Ramirez-Estrada K, Vidal-Limon H, Hidalgo D, et al. Elicitation, an Effective Strategy for the
625 Biotechnological Production of Bioactive High-Added Value Compounds in Plant Cell Factories. *Molecules*
626 2016; 21: 182.
- 627 [80] Newhouse T, Baran PS, Hoffmann RW. The economies of synthesis. *Chem Soc Rev* 2009; 38: 3010–3021.
- 628 [81] Andersen ØM, Markham KR (eds). *Flavonoids - Chemistry, Biochemistry and Applications*. Boca Raton,
629 USA: Taylor & Francis, 2006.

- 630 [82] Lee H, DeLoache WC, Dueber JE. Spatial organization of enzymes for metabolic engineering. *Metab Eng*
631 2012; 14: 242–251.
- 632 [83] Marienhagen J, Bott M. Metabolic engineering of microorganisms for the synthesis of plant natural
633 products. *J Biotechnol* 2013; 163: 166–178.
- 634 [84] Trantas EA, Koffas MAG, Xu P, et al. When plants produce not enough or at all: metabolic engineering of
635 flavonoids in microbial hosts. *Front Plant Sci* 2015; 6: 7.
- 636 [85] Hara KY, Araki M, Okai N, et al. Development of bio-based fine chemical production through synthetic
637 bioengineering. *Microb Cell Fact* 2014; 13: 173.
- 638 [86] Bicas JL, Molina G, Cavalcante Barros FF, et al. CHAPTER 12. Production of Aroma Compounds by White
639 Biotechnology. In: Coelho MAZ, Ribeiro BD (eds) *White Biotechnology for Sustainable Chemistry*.
640 Cambridge, UK: The Royal Society of Chemistry, pp. 310–332.
- 641 [87] Rawat I, Ranjith Kumar R, Mutanda T, et al. Biodiesel from microalgae: A critical evaluation from
642 laboratory to large scale production. *Appl Energy* 2013; 103: 444–467.
- 643 [88] Srirangan K, Akawi L, Moo-Young M, et al. Towards sustainable production of clean energy carriers from
644 biomass resources. *Appl Energy* 2012; 100: 172–186.
- 645 [89] Donnez D, Jeandet P, Clément C, et al. Bioproduction of resveratrol and stilbene derivatives by plant cells
646 and microorganisms. *Trends Biotechnol* 2009; 27: 706–713.
- 647 [90] Beekwilder J, van der Meer IM, Sibbesen O, et al. Microbial production of natural raspberry ketone.
648 *Biotechnol J* 2007; 2: 1270–1279.
- 649 [91] Zhang H, Stephanopoulos G. Engineering *E. coli* for caffeic acid biosynthesis from renewable sugars. *Appl*
650 *Microbiol Biotechnol* 2013; 97: 3333–3341.
- 651 [92] Wang S, Zhang S, Xiao A, et al. Metabolic engineering of *Escherichia coli* for the biosynthesis of various
652 phenylpropanoid derivatives. *Metab Eng* 2015; 29: 153–159.

- 653 [93] Katsuyama Y, Hirose Y, Funa N, et al. Precursor-directed biosynthesis of curcumin analogs in *Escherichia*
654 *coli*. *Biosci Biotechnol Biochem* 2010; 74: 641–645.
- 655 [94] Rodrigues JL, Araújo RG, Prather KLJ, et al. Production of curcuminoids from tyrosine by a metabolically
656 engineered *Escherichia coli* using caffeic acid as an intermediate. *Biotechnol J* 2015; 10: 599–609.
- 657 [95] Katz M, Durhuus T, Smits HP, et al. *Production of metabolites*. WO2011147818, European
658 Union <http://www.google.is/patents/WO2011147818A2?cl=is> (2011).
- 659 [96] Ananga A, Georgiev V, Tsoleva V. Manipulation and Engineering of Metabolic and Biosynthetic Pathway
660 of Plant Polyphenols. *Curr Pharm Des* 2013; 19: 6186–6206.
- 661 [97] Dixon R a, Liu C, Jun JH. Metabolic engineering of anthocyanins and condensed tannins in plants. *Curr*
662 *Opin Biotechnol* 2013; 24: 329–335.
- 663 [98] Korkina L, Kostyuk V. Biotechnologically produced secondary plant metabolites for cancer treatment and
664 prevention. *Curr Pharm Biotechnol* 2012; 13: 265–275.
- 665 [99] Giri CC, Zaheer M. Chemical elicitors versus secondary metabolite production in vitro using plant cell,
666 tissue and organ cultures: recent trends and a sky eye view appraisal. *Plant Cell, Tissue Organ Cult* 2016;
667 126: 1–18.
- 668 [100] Ro D-K, Douglas CJ. Reconstitution of the entry point of plant phenylpropanoid metabolism in yeast
669 (*Saccharomyces cerevisiae*): implications for control of metabolic flux into the phenylpropanoid pathway. *J*
670 *Biol Chem* 2004; 279: 2600–2607.
- 671 [101] Jendresen CB, Stahlhut SG, Li M, et al. Highly Active and Specific Tyrosine Ammonia-Lyases from
672 Diverse Origins Enable Enhanced Production of Aromatic Compounds in Bacteria and *Saccharomyces*
673 *cerevisiae*. *Appl Environ Microbiol* 2015; 81: 4458–76.
- 674 [102] Hwang E Il, Kaneko M, Ohnishi Y, et al. Production of Plant-Specific Flavanones by *Escherichia coli*
675 Containing an Artificial Gene Cluster. *Appl Environ Microbiol* 2003; 69: 2699–2706.

- 676 [103] Watts KT, Lee PC, Schmidt-Dannert C. Exploring recombinant flavonoid biosynthesis in metabolically
677 engineered *Escherichia coli*. *Chembiochem* 2004; 5: 500–507.
- 678 [104] Wang Y, Halls C, Zhang J, et al. Stepwise increase of resveratrol biosynthesis in yeast *Saccharomyces*
679 *cerevisiae* by metabolic engineering. *Metab Eng* 2011; 13: 455–463.
- 680 [105] Lin Y, Jain R, Yan Y. Microbial production of antioxidant food ingredients via metabolic engineering. *Curr*
681 *Opin Biotechnol* 2014; 26: 71–78.
- 682 [106] van Summeren-Wesenhagen P V, Marienhagen J. Metabolic engineering for phenylpropanoid-derived
683 products in microorganisms. *Bioengineered* 2013; 4: 355–362.
- 684 [107] Trantas E, Panopoulos N, Ververidis F. Metabolic engineering of the complete pathway leading to
685 heterologous biosynthesis of various flavonoids and stilbenoids in *Saccharomyces cerevisiae*. *Metab Eng*
686 2009; 11: 355–366.
- 687 [108] Zhu S, Wu J, Du G, et al. Efficient Synthesis of Eriodictyol from L-Tyrosine in *Escherichia coli*. *Appl*
688 *Environ Microbiol* 2014; 80: 3072–3080.
- 689 [109] Yan Y, Huang L, Koffas M a G. Biosynthesis of 5-deoxyflavanones in microorganisms. *Biotechnol J* 2007;
690 2: 1250–1262.
- 691 [110] Leonard E, Yan Y, Lim KH, et al. Investigation of Two Distinct Flavone Synthases for Plant-Specific
692 Flavone Biosynthesis in *Saccharomyces cerevisiae*. *Appl Environ Microbiol* 2005; 71: 8241–8248.
- 693 [111] Lee H, Kim BG, Kim M, et al. Biosynthesis of Two Flavones, Apigenin and Genkwanin, in *Escherichia*
694 *coli*. *J Microbiol Biotechnol* 2015; 25: 1442–8.
- 695 [112] Leonard E, Koffas M a G. Engineering of artificial plant cytochrome P450 enzymes for synthesis of
696 isoflavones by *Escherichia coli*. *Appl Environ Microbiol* 2007; 73: 7246–7251.
- 697 [113] Katsuyama Y, Miyahisa I, Funa N, et al. One-pot synthesis of genistein from tyrosine by coincubation of
698 genetically engineered *Escherichia coli* and *Saccharomyces cerevisiae* cells. *Appl Microbiol Biotechnol*

699 2006; 73: 1143–1149.

700 [114] Miyahisa I, Funa N, Ohnishi Y, et al. Combinatorial biosynthesis of flavones and flavonols in *Escherichia*
701 *coli*. *Appl Microbiol Biotechnol* 2006; 71: 53–58.

702 [115] Stahlhut SG, Siedler S, Malla S, et al. Assembly of a novel biosynthetic pathway for production of the plant
703 flavonoid fisetin in *Escherichia coli*. *Metab Eng* 2015; 31: 84–93.

704 [116] Chemler J, Lock LT, Koffas MG, et al. Standardized biosynthesis of flavan-3-ols with effects on pancreatic
705 beta-cell insulin secretion. *Appl Microbiol Biotechnol* 2007; 77: 797–807.

706 [117] Yan Y, Li Z, Koffas M a G. High-yield anthocyanin biosynthesis in engineered *Escherichia coli*. *Biotechnol*
707 *Bioeng* 2008; 100: 126–140.

708 [118] Lim CG, Wong L, Bhan N, et al. Development of a Recombinant *Escherichia coli* Strain for Overproduction
709 of the Plant Pigment Anthocyanin. *Appl Environ Microbiol* 2015; 81: 6276–6284.

710 [119] Kim B-G, Sung SH, Chong Y, et al. Plant Flavonoid O-Methyltransferases: Substrate Specificity and
711 Application. *J Plant Biol* 2010; 53: 321–329.

712 [120] Regev-Shoshani G, Shoseyov O, Bilkis I, et al. Glycosylation of resveratrol protects it from enzymic
713 oxidation. *Biochem J* 2003; 374: 157–63.

714 [121] Parajuli P, Pandey RP, Trang NTH, et al. Synthetic sugar cassettes for the efficient production of flavonol
715 glycosides in *Escherichia coli*. *Microb Cell Fact* 2015; 14: 76.

716 [122] He X-Z, Li W-S, Blount JW, et al. Regioselective synthesis of plant (iso)flavone glycosides in *Escherichia*
717 *coli*. *Appl Microbiol Biotechnol* 2008; 80: 253–260.

718 [123] Hirotani M, Kuroda R, Suzuki H, et al. Cloning and expression of UDP-glucose: flavonoid 7-O-
719 glucosyltransferase from hairy root cultures of *Scutellaria baicalensis*. *Planta* 2000; 210: 1006–1013.

720 [124] Choi SH, Ryu M, Yoon YJ, et al. Glycosylation of various flavonoids by recombinant oleandomycin

glycosyltransferase from *Streptomyces antibioticus* in batch and repeated batch modes. *Biotechnol Lett* 2012; 34: 499–505.

[125] Malla S, Pandey RP, Kim B-G, et al. Regiospecific modifications of naringenin for astragalin production in *Escherichia coli*. *Biotechnol Bioeng* 2013; 110: 2525–2535.

[126] Pandey RP, Parajuli P, Koirala N, et al. Glucosylation of Isoflavonoids in Engineered *Escherichia coli*. *Mol Cells* 2014; 37: 172–177.

[127] Li J, Li Z, Li C, et al. Molecular cloning and characterization of an isoflavone 7-O-glucosyltransferase from *Pueraria lobata*. *Plant Cell Rep* 2014; 33: 1173–1185.

[128] Yang S-M, Han SH, Kim B-G, et al. Production of kaempferol 3-O-rhamnoside from glucose using engineered *Escherichia coli*. *J Ind Microbiol Biotechnol* 2014; 41: 1311–8.

[129] Kim B-G, Kim HJ, Ahn J-H. Production of Bioactive Flavonol Rhamnosides by Expression of Plant Genes in *Escherichia coli*. *J Agric Food Chem* 2012; 60: 11143–11148.

[130] Pandey RP, Malla S, Simkhada D, et al. Production of 3-O-xylosyl quercetin in *Escherichia coli*. *Appl Microbiol Biotechnol* 2013; 97: 1889–1901.

[131] Simkhada D, Kim E, Lee HC, et al. Metabolic engineering of *Escherichia coli* for the biological synthesis of 7-O-xylosyl naringenin. *Mol Cells* 2009; 28: 397–401.

[132] Kim SY, Lee HR, Park K, et al. Metabolic engineering of *Escherichia coli* for the biosynthesis of flavonoid-O-glucuronides and flavonoid-O-galactoside. *Appl Microbiol Biotechnol* 2015; 99: 2233–2242.

[133] Kim B-G, Sung SH, Ahn J-H. Biological synthesis of quercetin 3-O-N-acetylglucosamine conjugate using engineered *Escherichia coli* expressing UGT78D2. *Appl Microbiol Biotechnol* 2012; 93: 2447–2453.

[134] Kim M-J, Kim B-G, Ahn J-H. Biosynthesis of bioactive O-methylated flavonoids in *Escherichia coli*. *Appl Microbiol Biotechnol* 2013; 97: 7195–7204.

743 [135] Malla S, Koffas MAG, Kazlauskas RJ, et al. Production of 7-O-methyl aromadendrin, a medicinally
744 valuable flavonoid, in *Escherichia coli*. *Appl Environ Microbiol* 2012; 78: 684–94.

745 [136] Becker J, Armstrong G, Vandermerwe M, et al. Metabolic engineering of for the synthesis of the wine-
746 related antioxidant resveratrol. *FEMS Yeast Res* 2003; 4: 79–85.

747 [137] Zhang Y, Li S-Z, Li J, et al. Using unnatural protein fusions to engineer resveratrol biosynthesis in yeast and
748 mammalian cells. *J Am Chem Soc* 2006; 128: 13030–1.

749 [138] Beekwilder J, Wolswinkel R, Jonker H, et al. Production of resveratrol in recombinant microorganisms.
750 *Appl Environ Microbiol* 2006; 72: 5670–5672.

751 [139] Watts KT, Lee PC, Schmidt-Dannert C. Biosynthesis of plant-specific stilbene polyketides in metabolically
752 engineered *Escherichia coli*. *BMC Biotechnol* 2006; 6: 22.

753 [140] Sydor T, Schaffer S, Boles E. Considerable increase in resveratrol production by recombinant industrial
754 yeast strains with use of rich medium. *Appl Environ Microbiol* 2010; 76: 3361–3363.

755 [141] Lim CG, Fowler ZL, Hueller T, et al. High-yield resveratrol production in engineered *Escherichia coli*. *Appl*
756 *Environ Microbiol* 2011; 77: 3451–3460.

757 [142] Choi O, Wu C-Z, Kang SY, et al. Biosynthesis of plant-specific phenylpropanoids by construction of an
758 artificial biosynthetic pathway in *Escherichia coli*. *J Ind Microbiol Biotechnol* 2011; 38: 1657–1665.

759 [143] Wu J, Liu P, Fan Y, et al. Multivariate modular metabolic engineering of *Escherichia coli* to produce
760 resveratrol from L-tyrosine. *J Biotechnol* 2013; 167: 404–411.

761 [144] Li M, Kildegaard KR, Chen Y, et al. De novo production of resveratrol from glucose or ethanol by
762 engineered *Saccharomyces cerevisiae*. *Metab Eng* 2015; 32: 1–11.

763 [145] Katsuyama Y, Funa N, Horinouchi S. Precursor-directed biosynthesis of stilbene methyl ethers in
764 *Escherichia coli*. *Biotechnol J* 2007; 2: 1286–1293.

765 [146] van Summeren-Wesenhagen P V, Marienhagen J. Metabolic Engineering of *Escherichia coli* for the
766 Synthesis of the Plant Polyphenol Pinosylvin. *Appl Environ Microbiol* 2015; 81: 840–849.

767 [147] Fulda S. Resveratrol and derivatives for the prevention and treatment of cancer. *Drug Discov Today* 2010;
768 15: 757–765.

769 [148] Wang Y, Bhuiya MW, Zhou R, et al. Pterostilbene production by microorganisms expressing resveratrol O-
770 methyltransferase. *Ann Microbiol* 2015; 65: 817–826.

771 [149] Jeong YJ, An CH, Woo SG, et al. Production of pinostilbene compounds by the expression of resveratrol O-
772 methyltransferase genes in *Escherichia coli*. *Enzyme Microb Technol* 2014; 54: 8–14.

773 [150] Huang Q, Lin Y, Yan Y. Caffeic acid production enhancement by engineering a phenylalanine over-
774 producing *Escherichia coli* strain. *Biotechnol Bioeng* 2013; 110: 3188–3196.

775 [151] Lin Y, Yan Y. Biosynthesis of caffeic acid in *Escherichia coli* using its endogenous hydroxylase complex.
776 *Microb Cell Fact* 2012; 11: 42.

777 [152] Kang S-Y, Choi O, Lee JK, et al. Artificial biosynthesis of phenylpropanoic acids in a tyrosine
778 overproducing *Escherichia coli* strain. *Microb Cell Fact* 2012; 11: 153.

779 [153] Lin Y, Sun X, Yuan Q, et al. Combinatorial biosynthesis of plant-specific coumarins in bacteria. *Metab Eng*
780 2013; 18: 69–77.

781 [154] Trenor SR, Shultz AR, Love BJ, et al. Coumarins in Polymers: From Light Harvesting to Photo-Cross-
782 Linkable Tissue Scaffolds. *Chem Rev* 2004; 104: 3059–3078.

783 [155] Lino CS, Taveira ML, Viana GSB, et al. Analgesic and antiinflammatory activities of *Justicia pectoralis*
784 Jacq and its main constituents: coumarin and umbelliferone. *Phyther Res* 1997; 11: 211–215.

785 [156] Park SR, Yoon JA, Paik JH, et al. Engineering of plant-specific phenylpropanoids biosynthesis in
786 *Streptomyces venezuelae*. *J Biotechnol* 2009; 141: 181–188.

787 [157] Park SR, Paik JH, Ahn MS, et al. Biosynthesis of plant-specific flavones and flavonols in *Streptomyces*
788 *venezuelae*. *J Microbiol Biotechnol* 2010; 20: 1295–9.

789 [158] Kallscheuer N, Vogt M, Stenzel A, et al. Construction of a *Corynebacterium glutamicum* platform strain for
790 the production of stilbenes and (2S)-flavanones. *Metab Eng* 2016; 38: 47–55.

791 [159] Kallscheuer N, Vogt M, Marienhagen J. A Novel Synthetic Pathway Enables Microbial Production of
792 Polyphenols Independent from the Endogenous Aromatic Amino Acid Metabolism. *ACS Synth Biol* 2016;
793 acssynbio.6b00291.

794 [160] Kang L, Li Q, Lin J, et al. Biosynthesis of Resveratrol in Blastospore of the Macrofungus *Tremella*
795 *fuciformis*. *Mol Biotechnol* 2015; 57: 675–684.

796 [161] Yang Y-T, Bennett GN, San K-Y. Genetic and metabolic engineering. *Electron J Biotechnol* 1998; 1: 134–
797 141.

798 [162] Bailey JE. Toward a science of metabolic engineering. *Science* 1991; 252: 1668–75.

799 [163] Yadav VG, De Mey M, Giaw Lim C, et al. The future of metabolic engineering and synthetic biology:
800 towards a systematic practice. *Metab Eng* 2012; 14: 233–41.

801 [164] Miyahisa I, Kaneko M, Funa N, et al. Efficient production of (2S)-flavanones by *Escherichia coli* containing
802 an artificial biosynthetic gene cluster. *Appl Microbiol Biotechnol* 2005; 68: 498–504.

803 [165] Fowler ZL, Gikandi WW, Koffas MAG. Increased malonyl coenzyme A biosynthesis by tuning the
804 *Escherichia coli* metabolic network and its application to flavanone production. *Appl Environ Microbiol*
805 2009; 75: 5831.

806 [166] Zha W, Rubin-Pitel SB, Shao Z, et al. Improving cellular malonyl-CoA level in *Escherichia coli* via
807 metabolic engineering. *Metab Eng* 2009; 11: 192–198.

808 [167] Leonard E, Lim K-H, Saw P-N, et al. Engineering central metabolic pathways for high-level flavonoid
809 production in *Escherichia coli*. *Appl Environ Microbiol* 2007; 73: 3877–3886.

- 810 [168] Wu J, Du G, Zhou J, et al. Metabolic engineering of *Escherichia coli* for (2S)-pinocembrin production from
811 glucose by a modular metabolic strategy. *Metab Eng* 2013; 16: 48–55.
- 812 [169] Leonard E, Yan Y, Fowler ZL, et al. Strain improvement of recombinant *Escherichia coli* for efficient
813 production of plant flavonoids. *Mol Pharm* 2008; 5: 257–65.
- 814 [170] Cao W, Ma W, Zhang B, et al. Improved pinocembrin production in *Escherichia coli* by engineering fatty
815 acid synthesis. *J Ind Microbiol Biotechnol* 2016; 43: 557–566.
- 816 [171] Liang J, Guo L, Lin J, et al. A novel process for obtaining pinosylvin using combinatorial bioengineering in
817 *Escherichia coli*. *World J Microbiol Biotechnol* 2016; 32: 102.
- 818 [172] Xu P, Ranganathan S, Fowler ZL, et al. Genome-scale metabolic network modeling results in minimal
819 interventions that cooperatively force carbon flux towards malonyl-CoA. *Metab Eng* 2011; 13: 578–587.
- 820 [173] Bhan N, Xu P, Khalidi O, et al. Redirecting carbon flux into malonyl-CoA to improve resveratrol titers:
821 Proof of concept for genetic interventions predicted by OptForce computational framework. *Chem Eng Sci*
822 2013; 103: 109–114.
- 823 [174] Chemler J a., Fowler ZL, McHugh KP, et al. Improving NADPH availability for natural product
824 biosynthesis in *Escherichia coli* by metabolic engineering. *Metab Eng* 2010; 12: 96–104.
- 825 [175] Jones JA, Vernacchio VR, Sinkoe AL, et al. Experimental and computational optimization of an *Escherichia*
826 *coli* co-culture for the efficient production of flavonoids. *Metab Eng* 2016; 35: 55–63.
- 827 [176] Brazier-Hicks M, Edwards R. Metabolic engineering of the flavone-C-glycoside pathway using polypeptide
828 technology. *Metab Eng* 2013; 16: 11–20.
- 829 [177] Koopman F, Beekwilder J, Crimi B, et al. De novo production of the flavonoid naringenin in engineered
830 *Saccharomyces cerevisiae*. *Microb Cell Fact* 2012; 11: 155.
- 831 [178] Santos CNS, Koffas M, Stephanopoulos G. Optimization of a heterologous pathway for the production of
832 flavonoids from glucose. *Metab Eng* 2011; 13: 392–400.

833 [179] Rodriguez A, Kildegaard KR, Li M, et al. Establishment of a yeast platform strain for production of *p*-
834 coumaric acid through metabolic engineering of aromatic amino acid biosynthesis. *Metab Eng* 2015; 31:
835 181–188.

836 [180] Portnoy VA, Bezdan D, Zengler K. Adaptive laboratory evolution—harnessing the power of biology for
837 metabolic engineering. *Curr Opin Biotechnol* 2011; 22: 590–594.

838 [181] Dragosits M, Mattanovich D, Clomburg J, et al. Adaptive laboratory evolution – principles and applications
839 for biotechnology. *Microb Cell Fact* 2013; 12: 64.

840 [182] van Summeren-Wesenhagen P V, Voges R, Dennig A, et al. Combinatorial optimization of synthetic
841 operons for the microbial production of *p*-coumaryl alcohol with *Escherichia coli*. *Microb Cell Fact* 2015;
842 14: 79.

843 [183] Siu K-H, Chen RP, Sun Q, et al. Synthetic scaffolds for pathway enhancement. *Curr Opin Biotechnol* 2015;
844 36: 98–106.

845 [184] Diaz E, Ferrández A, Prieto MA, et al. Biodegradation of Aromatic Compounds by *Escherichia coli*.
846 *Microbiol Mol Biol Rev* 2001; 65: 523–569.

847 [185] Jiang H, Wood K V, Morgan JA. Metabolic engineering of the phenylpropanoid pathway in *Saccharomyces*
848 *cerevisiae*. *Appl Environ Microbiol* 2005; 71: 2962–9.

849 [186] Kallscheuer N, Vogt M, Kappelmann J, et al. Identification of the *phd* gene cluster responsible for
850 phenylpropanoid utilization in *Corynebacterium glutamicum*. *Appl Microbiol Biotechnol* 2016; 100: 1871–
851 1881.

852 [187] Alper H, Stephanopoulos G. Uncovering the gene knockout landscape for improved lycopene production in
853 *E. coli*. *Appl Microbiol Biotechnol* 2008; 78: 801–810.

854 [188] Dietrich J a, McKee AE, Keasling JD. *High-throughput metabolic engineering: advances in small-molecule*
855 *screening and selection*. Epub ahead of print 2010. DOI: 10.1146/annurev-biochem-062608-095938.

- 856 [189] Siedler S, Stahlhut SG, Malla S, et al. Novel biosensors based on flavonoid-responsive transcriptional
857 regulators introduced into *Escherichia coli*. *Metab Eng* 2014; 21: 2–8.
- 858 [190] Xu P, Wang W, Li L, et al. Design and Kinetic Analysis of a Hybrid Promoter–Regulator System for
859 Malonyl-CoA Sensing in *Escherichia coli*. Epub ahead of print 2013. DOI: 10.1021/cb400623m.
- 860 [191] Liu D, Xiao Y, Evans BS, et al. Negative Feedback Regulation of Fatty Acid Production Based on a
861 Malonyl-CoA Sensor–Actuator. *ACS Synth Biol* 2015; 4: 132–140.
- 862 [192] Wang Z, Cirino PC. New and improved tools and methods for enhanced biosynthesis of natural products in
863 microorganisms. *Curr Opin Biotechnol* 2016; 42: 159–168.
- 864 [193] Rabausch U, Juergensen J, Ilmberger N, et al. Functional screening of metagenome and genome libraries for
865 detection of novel flavonoid-modifying enzymes. *Appl Environ Microbiol* 2013; 79: 4551–4563.
- 866 [194] Galati G, O’Brien PJ. Potential toxicity of flavonoids and other dietary phenolics: significance for their
867 chemopreventive and anticancer properties. *Free Radic Biol Med* 2004; 37: 287–303.
- 868 [195] Chlopčíková Š, Psotová J, Miketová P, et al. Chemoprotective effect of plant phenolics against
869 anthracycline-induced toxicity on rat cardiomyocytes Part II. caffeic, chlorogenic and rosmarinic acids.
870 *Phyther Res* 2004; 18: 408–413.
- 871 [196] Berrougui H, Cloutier M, Isabelle M, et al. Phenolic-extract from argan oil (*Argania spinosa* L.) inhibits
872 human low-density lipoprotein (LDL) oxidation and enhances cholesterol efflux from human THP-1
873 macrophages. *Atherosclerosis* 2006; 184: 389–396.
- 874 [197] Wilson TA, Nicolosi RJ, Woolfrey B, et al. Rice bran oil and oryzanol reduce plasma lipid and lipoprotein
875 cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in
876 hypercholesterolemic hamsters. *J Nutr Biochem* 2007; 18: 105–112.
- 877 [198] Dembinska-Kiec A, Mykkänen O, Kiec-Wilk B, et al. Antioxidant phytochemicals against type 2 diabetes.
878 *Br J Nutr* 2008; 99: ES109-ES117.

- 879 [199] Ferruzzi MG, Lobo JK, Janle EM, et al. Bioavailability of Gallic Acid and Catechins from Grape Seed
880 Polyphenol Extract is Improved by Repeated Dosing in Rats: Implications for Treatment in Alzheimer's
881 Disease. *J Alzheimer's Dis* 2009; 18: 113–124.
- 882 [200] El-Seedi HR, El-Said AM a, Khalifa S a M, et al. Biosynthesis, natural sources, dietary intake,
883 pharmacokinetic properties, and biological activities of hydroxycinnamic acids. *J Agric Food Chem* 2012;
884 60: 10877–10895.
- 885 [201] Graf E. Antioxidant potential of ferulic acid. *Free Radic Biol Med* 1992; 13: 435–448.
- 886 [202] Daglia M. Polyphenols as antimicrobial agents. *Curr Opin Biotechnol* 2012; 23: 174–181.
- 887 [203] Pyrzynska K, Biesaga M. Analysis of phenolic acids and flavonoids in honey. *TrAC Trends Anal Chem*
888 2009; 28: 893–902.
- 889 [204] Mahmood N, Moore PS, De Tommasi N, et al. Inhibition of HIV Infection by Caffeoylquinic Acid
890 Derivatives. *Antivir Chem Chemother* 1993; 4: 235–240.
- 891 [205] Del Rio D, Borges G, Crozier A. Berry flavonoids and phenolics: bioavailability and evidence of protective
892 effects. *Br J Nutr* 2010; 104 Suppl: S67-90.
- 893 [206] Nichenametla SN, Taruscio TG, Barney DL, et al. A Review of the Effects and Mechanisms of
894 Polyphenolics in Cancer. <http://dx.doi.org/10.1080/10408390591000541>.
- 895 [207] Wang L-S, Stoner GD. Anthocyanins and their role in cancer prevention. *Cancer Lett* 2008; 269: 281–290.
- 896 [208] Lila MA. Anthocyanins and Human Health: An In Vitro Investigative Approach. *J Biomed Biotechnol* 2004;
897 2004: 306–313.
- 898 [209] Alvarez-Suarez JM, Giampieri F, Tulipani S, et al. One-month strawberry-rich anthocyanin supplementation
899 ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. *J Nutr Biochem*
900 2014; 25: 289–294.

901 [210] Tsuda T. Regulation of Adipocyte Function by Anthocyanins; Possibility of Preventing the Metabolic
902 Syndrome. *J Agric Food Chem* 2008; 56: 642–646.

903 [211] Galli RL, Sshukitt-Hale B, Youdim KA, et al. Fruit Polyphenolics and Brain Aging. *Ann N Y Acad Sci* 2002;
904 959: 128–132.

905 [212] Mandel S a, Amit T, Weinreb O, et al. Understanding the broad-spectrum neuroprotective action profile of
906 green tea polyphenols in aging and neurodegenerative diseases. *J Alzheimers Dis* 2011; 25: 187–208.

907 [213] Shipp J, Abdel-Aal E-SM. Food Applications and Physiological Effects of Anthocyanins as Functional Food
908 Ingredients. *Open Food Sci J* 2010; 4: 7–22.

909 [214] Moreno-Indias I, Sánchez-Alcoholado L, Pérez-Martínez P, et al. Red wine polyphenols modulate fecal
910 microbiota and reduce markers of the metabolic syndrome in obese patients. *Food Funct* 2016; 7: 1775–
911 1787.

912 [215] Suh Y, Afaq F, Johnson JJ, et al. A plant flavonoid fisetin induces apoptosis in colon cancer cells by
913 inhibition of COX2 and Wnt/EGFR/NF- B-signaling pathways. *Carcinogenesis* 2008; 30: 300–307.

914 [216] Liao Y-C, Shih Y-W, Chao C-H, et al. Involvement of the ERK Signaling Pathway in Fisetin Reduces
915 Invasion and Migration in the Human Lung Cancer Cell Line A549. *J Agric Food Chem* 2009; 57: 8933–
916 8941.

917 [217] Murakami A, Ashida H, Terao J. Multitargeted cancer prevention by quercetin. *Cancer Lett* 2008; 269: 315–
918 325.

919 [218] Zhou M, Ren H, Han J, et al. Protective Effects of Kaempferol against Myocardial Ischemia/Reperfusion
920 Injury in Isolated Rat Heart via Antioxidant Activity and Inhibition of Glycogen Synthase Kinase-3 β . *Oxid*
921 *Med Cell Longev* 2015; 2015: 481405.

922 [219] Calderon-Montano JM, Burgos-Moron E, Perez-Guerrero C, et al. A Review on the Dietary Flavonoid
923 Kaempferol. *Mini Rev Med Chem* 2011; 11: 298–344(47).

- 924 [220] Dajas F, Andrés A-CJ, Florencia A, et al. Neuroprotective actions of flavones and flavonols: mechanisms
925 and relationship to flavonoid structural features. *Cent Nerv Syst Agents Med Chem* 2013; 13: 30–35.
- 926 [221] Maher P. Modulation of multiple pathways involved in the maintenance of neuronal function during aging
927 by fisetin. *Genes Nutr* 2009; 4: 297–307.
- 928 [222] Currais A, Prior M, Dargusch R, et al. Modulation of p25 and inflammatory pathways by fisetin maintains
929 cognitive function in Alzheimer’s disease transgenic mice. *Aging Cell* 2014; 13: 379–390.
- 930 [223] Funakoshi-Tago M, Nakamura K, Tago K, et al. Anti-inflammatory activity of structurally related
931 flavonoids, Apigenin, Luteolin and Fisetin. *Int Immunopharmacol* 2011; 11: 1150–1159.
- 932 [224] Maher P, Akaishi T, Abe K. Flavonoid fisetin promotes ERK-dependent long-term potentiation and
933 enhances memory. *Proc Natl Acad Sci* 2006; 103: 16568–16573.
- 934 [225] Raygude KS, Kandhare AD, Ghosh P, et al. Anticonvulsant effect of fisetin by modulation of endogenous
935 biomarkers. *Biomed Prev Nutr* 2012; 2: 215–222.
- 936 [226] Chung S Yang, Janelle M Landau, Mou-Tuan Huang A, et al. Inhibition of carcinogenesis by dietary
937 polyphenolic compounds. *Annu Rev Nutr* 2003; 21: 381–406.
- 938 [227] Islam M a. Cardiovascular effects of green tea catechins: progress and promise. *Recent Pat Cardiovasc*
939 *Drug Discov* 2012; 7: 88–99.
- 940 [228] Prasath GS, Pillai SI, Subramanian SP. Fisetin improves glucose homeostasis through the inhibition of
941 gluconeogenic enzymes in hepatic tissues of streptozotocin induced diabetic rats. *Eur J Pharmacol* 2014;
942 740: 248–254.
- 943 [229] Yoshino K, Hara Y, Sano M, et al. Antioxidative Effects of Black Tea Theaflavins and Thearubigin on Lipid
944 Peroxidation of Rat Liver Homogenates Induced by tert-Butyl Hydroperoxide. *Biol Pharm Bull* 1994; 17:
945 146–149.
- 946 [230] de Pascual-Teresa S, Moreno DA, García-Viguera C. Flavanols and Anthocyanins in Cardiovascular Health:

947 A Review of Current Evidence. *Int J Mol Sci* 2010; 11: 1679–1703.

948 [231] Ramassamy C. Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: A
949 review of their intracellular targets. *Eur J Pharmacol* 2006; 545: 51–64.

950 [232] Miyamoto K, Murayama T, Nomura M, et al. Antitumor activity and interleukin-1 induction by tannins.
951 *Anticancer Res* 1992; 13: 37–42.

952 [233] Adams LS, Zhang Y, Seeram NP, et al. Pomegranate Ellagitannin-Derived Compounds Exhibit
953 Antiproliferative and Antiaromatase Activity in Breast Cancer Cells In vitro. *Cancer Prev Res* 2010; 3: 108–
954 113.

955 [234] Yuan T, Ding Y, Wan C, et al. Antidiabetic Ellagitannins from Pomegranate Flowers: Inhibition of α -
956 Glucosidase and Lipogenic Gene Expression.

957 [235] Larrosa M, García-Conesa MT, Espín JC, et al. Ellagitannins, ellagic acid and vascular health. *Mol Aspects*
958 *Med* 2010; 31: 513–539.

959 [236] Arapitsas P. Hydrolyzable tannin analysis in food. *Food Chem* 2012; 135: 1708–1717.

960 [237] Machado T de B, Leal ICR, Amaral ACF, et al. Antimicrobial Ellagitannin of *Punica granatum* Fruits. *J*
961 *Braz Chem Soc* 2002; 13: 606–610.

962 [238] H. Sarkar F, Li Y, Wang Z, et al. Lesson Learned from Nature for the Development of Novel Anti-Cancer
963 Agents: Implication of Isoflavone, Curcumin, and their Synthetic Analogs. *Curr Pharm Des* 2010; 16:
964 1801–1812.

965 [239] Sarkar FH, Adsule S, Padhye S, et al. The Role of Genistein and Synthetic Derivatives of Isoflavone in
966 Cancer Prevention and Therapy. *Mini Rev Med Chem* 2006; 6: 401–407.

967 [240] Kondo K, Suzuki Y, Ikeda Y, et al. Genistein, an isoflavone included in soy, inhibits thrombotic vessel
968 occlusion in the mouse femoral artery and in vitro platelet aggregation. *Eur J Pharmacol* 2002; 455: 53–57.

- 969 [241] Kim JM, Yun-Choi HS. Anti-platelet effects of flavonoids and flavonoid-glycosides from *Sophora japonica*.
970 *Arch Pharm Res* 2008; 31: 886–890.
- 971 [242] Divi RL, Chang HC, Doerge DR. Anti-Thyroid Isoflavones from Soybean: Isolation, Characterization, and
972 Mechanisms of Action. *Biochem Pharmacol* 1997; 54: 1087–1096.
- 973 [243] Han KK, Soares JMJ, Haidar MA, et al. Benefits of soy isoflavone therapeutic regimen on menopausal
974 symptoms. *Obstet Gynecol* 2002; 99: 389–94.
- 975 [244] Lee Y-B, Lee HJ, Sohn HS. Soy isoflavones and cognitive function. *J Nutr Biochem* 2005; 16: 641–649.
- 976 [245] Koleckar V, Kubikova K, Rehakova Z, et al. Condensed and hydrolysable tannins as antioxidants
977 influencing the health. *MINI-REVIEWS Med Chem* 2008; 8: 436–447.
- 978 [246] Nandakumar V, Singh T, Katiyar SK. Multi-targeted prevention and therapy of cancer by
979 proanthocyanidins. *Cancer Lett* 2008; 269: 378–387.
- 980 [247] Yamakoshi J, Kataoka S, Koga T, et al. Proanthocyanidin-rich extract from grape seeds attenuates the
981 development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis* 1999; 142: 139–149.
- 982 [248] Sato M, Maulik G, Ray PS, et al. Cardioprotective Effects of Grape Seed Proanthocyanidin Against
983 Ischemic Reperfusion Injury. *J Mol Cell Cardiol* 1999; 31: 1289–1297.
- 984 [249] Kamimura A, Takahashi T. Procyanidin B-3, isolated from barley and identified as a hair-growth stimulant,
985 has the potential to counteract inhibitory regulation by TGF-beta1. *Exp Dermatol* 2002; 11: 532–541.
- 986 [250] Baek N-I, Chung M-S, Shamon L, et al. Selliguelain A, a Novel Highly Sweet Proanthocyanidin from the
987 Rhizomes of *Selliguea feei*. *J Nat Prod* 1993; 56: 1532–1538.
- 988 [251] Mukherjee M, Bandyopadhyay P, Kundu D. Exploring the role of cranberry polyphenols in periodontitis: A
989 brief review. *J Indian Soc Periodontol* 2014; 18: 136–9.
- 990 [252] Adlercreutz H. *Lignans and human health*. Epub ahead of print 2007. DOI: 10.1080/10408360701612942.

- 991 [253] Fini L, Hotchkiss E, Fogliano V, et al. Chemopreventive properties of pinorexinol-rich olive oil involve a
 992 selective activation of the ATM-p53 cascade in colon cancer cell lines. *Carcinogenesis* 2007; 29: 139–146.
- 993 [254] Canel C, Moraes RM, Dayan FE, et al. Podophyllotoxin. *Phytochemistry* 2000; 54: 115–120.
- 994 [255] Peterson J, Dwyer J, Adlercreutz H, et al. Dietary lignans: physiology and potential for cardiovascular
 995 disease risk reduction. *Nutr Rev* 2010; 68: 571–603.
- 996 [256] Xu Z, Ju J, Wang K, et al. Evaluation of hypoglycemic activity of total lignans from *Fructus arctii* in the
 997 spontaneously diabetic Goto-Kakizaki rats. *J Ethnopharmacol* 2014; 151: 548–555.
- 998 [257] Miyazawa M, Utsunomiya H, Inada K, et al. Inhibition of *Helicobacter pylori* Motility by (+)-
 999 Syringaresinol from Unripe Japanese Apricot. *Biol Pharm Bull* 2006; 29: 172–173.
- 1000 [258] Rimando AM, Cuendet M, Desmarchelier C, et al. Cancer Chemopreventive and Antioxidant Activities of
 1001 Pterostilbene, a Naturally Occurring Analogue of Resveratrol. *J Agric Food Chem* 2002; 50: 3453–3457.
- 1002 [259] Du G, Sun L, Zhao R, et al. Polyphenols: Potential source of drugs for the treatment of ischaemic heart
 1003 disease. *Pharmacol Ther* 2016; 162: 23–34.
- 1004 [260] Brasnyó P, Molnár GA, Mohás M, et al. Resveratrol improves insulin sensitivity, reduces oxidative stress
 1005 and activates the Akt pathway in type 2 diabetic patients. *Br J Nutr* 2011; 106: 383–389.
- 1006 [261] Rimando AM, Nagmani R, Feller DR, et al. Pterostilbene, a New Agonist for the Peroxisome Proliferator-
 1007 Activated Receptor α -Isoform, Lowers Plasma Lipoproteins and Cholesterol in Hypercholesterolemic
 1008 Hamsters. *J Agric Food Chem* 2005; 53: 3403–3407.
- 1009 [262] Szkudelska K, Szkudelski T. Resveratrol, obesity and diabetes. *Eur J Pharmacol* 2010; 635: 1–8.
- 1010 [263] Paredes-López O, Cervantes-Ceja ML, Vigna-Pérez M, et al. Berries: improving human health and healthy
 1011 aging, and promoting quality life - a review. *Plant Foods Hum Nutr* 2010; 65: 299–308.
- 1012 [264] Duan G-L, Wang C-N, Liu Y-J, et al. Resveratrol alleviates endotoxemia-associated adrenal insufficiency

1013 by suppressing oxidative/nitrative stress. *Endocr J* 2016; 63: 569–580.

1014 [265] Ling K-H, Wan MLY, El-Nezami H, et al. Protective Capacity of Resveratrol, a Natural Polyphenolic
1015 Compound, against Deoxynivalenol-Induced Intestinal Barrier Dysfunction and Bacterial Translocation.
1016 *Chem Res Toxicol* 2016; 29: 823–833.

1017 [266] Remsberg CM, Yáñez JA, Ohgami Y, et al. Pharmacometrics of pterostilbene: preclinical pharmacokinetics
1018 and metabolism, anticancer, antiinflammatory, antioxidant and analgesic activity. *Phyther Res* 2008; 22:
1019 169–179.

1020 [267] Yan Y, Chemler J, Huang L, et al. Metabolic Engineering of Anthocyanin Biosynthesis in *Escherichia coli*.
1021 *Appl Environ Microbiol* 2005; 71: 3617–3623.

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1024 **Table 1. Health-beneficial properties of the major groups of polyphenols.**

| Polyphenol | Applications: health-promoting or biotechnological |
|--|--|
| Phenolic acids <i>Examples:</i> Gallic acid Caffeic acid Ferulic acid Chlorogenic acid | Cancer: Chemopreventive activity, as well as protection against side effects of chemotherapy [194, 195]. CVD and Diabetes: Prevent oxidation of low-density lipoprotein (LDL)-cholesterol and effective in the treatment of hypercholesterolemia and type 2 diabetes [196–198]. Neurodegenerative diseases: Potential as agents for the treatment of Alzheimer's disease [199]. Others: Anti-allergic; anti-microbial; antioxidant and immunomodulatory activities. Di- and tri-caffeic/quinic acids have antiretroviral activity [200–204]. |
| Anthocyanins <i>Examples:</i> Cyanidin Pelargonidin Delphinidin Rosinidin | Cancer: Inhibit initiation and progression stages of tumor development; reduce effect of inflammation on promotion of tumorigenesis; suppress angiogenesis; minimize cancer-induced DNA damage [205–207]. CVD and Diabetes: Improve vascular health; protect against cardiovascular diseases; anti-obesity effects through improvement of adipocyte function; may contribute to prevention of the metabolic syndrome; potential anti-diabetic activity [64, 205, 208–210]. Neurodegenerative diseases: Protection against brain ageing and decline in cognitive performance in animal models [205, 211, 212]. Others: Reduce inflammatory biomarkers; bacteriostatic against some gut pathogenic bacteria; food colorants [205, 208, 213, 214]. |
| Flavonols <i>Examples:</i> Quercetin Kaempferol Myricetin Fisetin Morin | Cancer: Protective effects against pancreatic, breast, cervical, prostate, uterine, urinary tract cancers, and leukemia [49, 215–217]. CVD and Diabetes: Confer cardioprotection and improve the levels of risk factors for cardiovascular disease [218, 219]. Neurodegenerative diseases: Neuroprotective activity in experimental focal ischemia and models of neurodegeneration; cognition-enhancing; reduce the risk of Alzheimer's disease [220–222]. |

| | |
|--|---|
| Rutin | <p>Inflammation: Anti-inflammatory [223].</p> <p>Others: Anticonvulsant, antioxidants, memory enhancement [203, 224, 225].</p> |
| <p>Flavanols</p> <p><i>Examples:</i></p> <p>Catechins</p> <p>Epigallocatechin</p> <p>Thearubigins</p> <p>Mesquitol</p> | <p>Cancer: Inhibition of tumorigenesis in different organs of animals [205, 226].</p> <p>CVD and Diabetes: Cardioprotective effect by reverting of endothelial dysfunctions; decreasing inflammatory biomarkers, and providing antioxidant and antiplatelet effects. Also have beneficial effects on blood pressure, blood glucose level, and lipid parameters [227–230].</p> <p>Neurodegenerative diseases: Neuroprotective/neuroregenerative effects as modulators of intracellular neuronal signaling and metabolism, cell survival/death genes, and mitochondrial function [212, 231].</p> |
| <p>Hydrolyzable tannins</p> <p><i>Examples:</i></p> <p>Grandinin</p> <p>Casuarictin</p> <p>Punicalagin</p> <p>Vescalagin</p> | <p>Cancer: Anti-tumor, anti-proliferative and anti-mutagenic effects [232, 233].</p> <p>CVD and Diabetes: Anti-diabetic; anti-atherogenic; anti-thrombotic [234, 235].</p> <p>Others: Anti-inflammatory, anti-bacterial, and anti-mycotic properties. Ellagitannins and gallotannins may also affect the life of foodstuff due to their antioxidant properties and/or antimicrobial activity [236, 237].</p> |
| <p>Isoflavones</p> <p><i>Examples:</i></p> <p>Genistein</p> <p>Daidzein</p> <p>Curcumin</p> <p>Glycetin</p> | <p>Cancer: Inhibition of cell proliferation [47, 238, 239].</p> <p>CVD and Diabetes: Anti-platelet effects [240, 241].</p> <p>Others: Neuroprotective agents; improve cognitive functions and alleviate menopause symptom in females; anti-thyroid activity [242–244].</p> |
| <p>Proanthocyanidins</p> <p>(Condensed tannins)</p> <p><i>Examples:</i></p> <p>Epicatechin trimer</p> <p>Selliguesin A</p> <p>Procyanidin B3</p> | <p>Cancer: Reduce the incidence and progression of cancer (particularly of prostate cancer) [245, 246].</p> <p>CVD and Diabetes: Reduction of CVD incidence due to their antioxidant activity; inhibition of LDL oxidation; vasodilating properties; anti-platelet activity and protection against ischemia-reperfusion injury [245, 247, 248].</p> <p>Others: Proanthocyanidin-rich extracts inhibit viral adhesion and infectivity of the A</p> |

| | |
|--|--|
| | and B influenza viruses, as well as suppress urinary and <i>Helicobacter pylori</i> infections, procyanidin B3 has been described as a hair-growth stimulant, selliguelain A is a natural sweetener [245, 249–251]. |
| Lignans <i>Examples:</i> Secoisolariciresinol Pinoresinol Podophyllotoxin Steganacin | Cancer: Anti-carcinogenic effects on multiple types of cancer [252–254]. CVD and Diabetes: Associated with a decreased risk of cardiovascular diseases, hypoglycemic properties [255, 256]. Others: Inhibition of <i>H. pylori</i> motility and steroid hormone metabolism, anti-viral activities [252, 254, 257]. |
| Stilbenoids <i>Examples:</i> Resveratrol Pterostilbene Pinosylvin Piceid | Cancer: <i>in vitro</i> as well as <i>in vivo</i> chemopreventive and chemotherapeutic activities, in all three stages of carcinogenesis (initiation, promotion, and progression) [46, 258]. CVD and Diabetes: Improve insulin sensitivity, mimics calorie restriction, lower plasma lipoproteins and cholesterol, prevent cell damage induced by oxidative stress and ischemia [259–262]. Others: anti-aging and anti-inflammatory activities, relieves endotoxemia-associated adrenocortical insufficiency, confer protection against intestinal barrier dysfunction, modulate gut microbiota by favoring increase in lactic acid bacteria counts [46, 214, 263–266]. |

1025 CVD – cardiovascular diseases

1026

1027 **Table 2. Examples of polyphenol-containing products accessible to consumers.**

| Type of polyphenol | Production method | Examples of products on the market (supplier) |
|---|--|---|
| Phenolic acids (Hydroxycinnamic and hydroxybenzoic acids) | <ul style="list-style-type: none"> • Extraction from plants | <ul style="list-style-type: none"> • GCA™-Green Coffee Antioxidant (Applied Food Sciences) |
| Anthocyanins | <ul style="list-style-type: none"> • Extraction from plants • Extraction from bilberry • Extraction from bilberry | <ul style="list-style-type: none"> • Freeze Dried Polyphenol Fruitbasket (BerryPharma) • Mirtoselect® and Myrtocyan® (Indena®) • NutriPhy® Bilberry 100 (Chr. Hansen A/S) |
| Flavonols (Quercetin/kaempferol/myricetin) | <ul style="list-style-type: none"> • Chemical synthesis • Extraction from plants | <ul style="list-style-type: none"> • Quercetin complex (Solgar) • Bayberry Bark Extract Myricetin (Cactus Botanics) |
| Flavanols (Catechins) | <ul style="list-style-type: none"> • Extraction from plants • Extraction from plants • Extraction from plants | <ul style="list-style-type: none"> • Green Tea Catechins, Decaf - <i>Camellia sinensis</i>, (Amax) • NutraSource, AssuriTEA Green, (Kemin Health) • Theaflavin Black Tea Extract (Applied Food Sciences) |
| Hydrolyzable tannins (Casuarictin) | <ul style="list-style-type: none"> • Extraction from plants | <ul style="list-style-type: none"> • PomActiv™ Pomegranate Extract (Cyvex Nutrition) |
| Isoflavones (Genistein) | <ul style="list-style-type: none"> • Extraction from soy | <ul style="list-style-type: none"> • geniVida® (DSM) |
| Proanthocyanidins (Epichatechin trimer) | <ul style="list-style-type: none"> • Extraction from plants | <ul style="list-style-type: none"> • Pine Bark 95% Proanthocyanidins (Cactus Botanics) |

| | | |
|--|--|--|
| | <ul style="list-style-type: none"> • Extraction from plants | <ul style="list-style-type: none"> • ENOVITA® - grape seed extract and proanthocyanidin A2 phytosome (Indena) |
| Lignans (Secoisolariciresinol) | <ul style="list-style-type: none"> • Extraction from plants • Extraction from plants | <ul style="list-style-type: none"> • Flaxseed Lignans (Cactus Botanics) • ActiFlax (Marco Hi-Tech) |
| Stilbenes (Resveratrol) | <ul style="list-style-type: none"> • Extraction from plants • Chemical synthesis • Microbial production | <ul style="list-style-type: none"> • Rexatrol® - resveratrol phytosome® (Indena) • ResVida (DSM) • EveResveratrol™ (Evolva) |

1028

1029

1030 **Table 3. Production of polyphenolic compounds in microbial hosts.**

| Compound | Precursor | Host | Reference | Highest titer |
|-------------------------|-------------------------|-----------------------|-----------------|----------------|
| Phenolic acids | | | | |
| <i>p</i> -coumaric acid | L-Phenylalanine | <i>S. cerevisiae</i> | [100] | ~ 7.2 mg/L |
| | L-Tyrosine | <i>S. cerevisiae</i> | [179] | 1.93 g/L |
| | L-Tyrosine | <i>E. coli</i> | [101, 152] | 1.6 mmol/g CDW |
| | | <i>S. cerevisiae</i> | [101] | 133 µmol/g CDW |
| | | <i>L. lactis</i> | | 43 µmol/g CDW |
| Caffeic acid | L-Tyrosine | <i>E. coli</i> | [151, 152] | 150 mg/L |
| | Glucose | <i>E. coli</i> | [91, 150] | 767 mg/L |
| Ferulic acid | L-Tyrosine | <i>E. coli</i> | [152] | 196 mg/L |
| Flavanones | | | | |
| Naringenin | L-Tyrosine | <i>E. coli</i> | [102, 103, 164] | 57 mg/L |
| | L-Phenylalanine | <i>S. cerevisiae</i> | [107] | 8.9 mg/L |
| | <i>p</i> -Coumaric acid | <i>E. coli</i> | [172] | 474 mg/L |
| | Glucose | <i>E. coli</i> | [178] | 84 mg/L |
| | Glucose | <i>C. glutamicum</i> | [158] | 32 mg/L |
| | Glucose | <i>S. cerevisiae</i> | [177] | 113 mg/L |
| | <i>p</i> -Coumaric acid | <i>St. venezuelae</i> | [156] | 4 mg/L |
| Pinocembrin | L-Phenylalanine | <i>E. coli</i> | [102, 164] | 58 mg/L |
| | Glucose | <i>E. coli</i> | [168] | 40 mg/L |
| | Cinnamic acid | <i>St. venezuelae</i> | [156] | 6 mg/L |
| Eriodictyol | L-Tyrosine | <i>E. coli</i> | [108] | 43 mg/L |
| Liquiritigenin | <i>p</i> -Coumaric acid | <i>E. coli</i> | [109] | 17 mg/L |
| | | <i>S. cerevisiae</i> | | 14 mg/L |

| | | | | |
|--------------------|-------------------------|-----------------------|------------|-------------|
| 7-hydroxyflavanone | Cinnamic acid | <i>E. coli</i> | | 1.9 mg/L |
| | | <i>S. cerevisiae</i> | | 0.9 mg/L |
| Butin | Caffeic acid | <i>E. coli</i> | | 4.2 mg/L |
| | | <i>S. cerevisiae</i> | | 2.5 mg/L |
| Sakuranetin | L-Tyrosine | <i>E. coli</i> | [134] | 40 mg/L |
| Ponciretin | L-Tyrosine | <i>E. coli</i> | [134] | 43 mg/L |
| | | | | |
| Flavones | | | | |
| Chrysin | Cinnamic acid | <i>S. cerevisiae</i> | [110] | 0.9 mg/L |
| | L-Phenylalanine | <i>E. coli</i> | [114] | 9 mg/L |
| Apigenin | <i>p</i> -Coumaric acid | <i>S. cerevisiae</i> | [110] | 0.4 mg/L |
| | L-Tyrosine | <i>E. coli</i> | [111, 114] | 30 mg/L |
| Genkwanin | L-Tyrosine | <i>E. coli</i> | [111] | 41 mg/L |
| Luteolin | Caffeic acid | <i>S. cerevisiae</i> | [110] | 1.6 mg/L |
| | | | | |
| Isoflavones | | | | |
| Genistein | Naringenin | <i>E. coli</i> | [112] | 10 mg/g CDW |
| | L-Phenylalanine | <i>S. cerevisiae</i> | [107] | 0.1 mg/L |
| Daidzein | Liquiritigenin | <i>E. coli</i> | [112] | 18 mg/g CDW |
| | | | | |
| Flavonols | | | | |
| Kaempferol | L-Tyrosine | <i>E. coli</i> | [114] | 15 mg/L |
| | Naringenin | <i>St. venezuelae</i> | [157] | 0.2 mg/L |
| | L-Phenylalanine | <i>S. cerevisiae</i> | [107] | 1.3 mg/L |
| Galangin | L-Phenylalanine | <i>E. coli</i> | [114] | 1.1 mg/L |
| | Pinocembrin | <i>St. venezuelae</i> | [157] | 1.0 mg/L |
| Fisetin | L-Tyrosine | <i>E. coli</i> | [115] | 0.3 mg/L |

| | | | | |
|-------------------------------------|-------------------------|-----------------------|-----------------|--------------|
| Quercetin | <i>p</i> -Coumaric acid | <i>S. cerevisiae</i> | [107] | 0.26 mg/L |
| 7-O-methyl aromadendrin | <i>p</i> -Coumaric acid | <i>E. coli</i> | [135] | 3 mg/L |
| | | | | |
| Flavan-3-ol | | | | |
| (+)-catechin | Caffeic acid | <i>E. coli</i> | [116] | 0.09 mg/L |
| (+)-afzelechin | <i>p</i> -Coumaric acid | <i>E. coli</i> | [116] | 0.04 mg/L |
| | | | | |
| Anthocyanins | | | | |
| Pelargonidin 3- <i>O</i> -glucoside | Naringenin | <i>E. coli</i> | [267] | 6 µg/L |
| Cyanidin 3- <i>O</i> -glucoside | Eriodictyol | <i>E. coli</i> | [267] | 6 µg/L |
| | (+)-catechin | <i>E. coli</i> | [118] | 350 mg/L |
| | | | | |
| Stilbenes | | | | |
| Resveratrol | <i>p</i> -Coumaric acid | <i>S. cerevisiae</i> | [136–138, 140] | 391 mg/L |
| | Glucose | <i>S. cerevisiae</i> | [144] | 531 mg/L |
| | Glucose | <i>S. cerevisiae</i> | [95] | 4 g/L |
| | <i>p</i> -Coumaric acid | <i>E. coli</i> | [139, 141, 173] | 1600 mg/L |
| | Glucose | <i>C. glutamicum</i> | [158] | 59 mg/L |
| | <i>p</i> -Coumaric acid | <i>St. venezuelae</i> | [156] | 0.4 mg/L |
| | <i>p</i> -Coumaric acid | <i>T. fuciformis</i> | [160] | 0.8 µg/g CDW |
| Pinosylvin | L-Phenylalanine | <i>E. coli</i> | [145, 146] | 91 mg/L |
| | Glycerol | <i>E. coli</i> | [171] | 47 mg/L |
| | L-Phenylalanine | <i>St. venezuelae</i> | [156] | 0.6 mg/L |
| Pterostilbene | <i>p</i> -Coumaric acid | <i>E. coli</i> | [148] | 50 mg/L |
| Pinostilbene | <i>p</i> -Coumaric acid | <i>E. coli</i> | [149] | 34 mg/L |

1031 CDW – cell dry weight

1032 **Table 4: Metabolic engineering strategies used for improving precursor supply for polyphenol biosynthesis**

| Target | Approach | Host organism | References |
|---|--|----------------------|----------------------|
| Malonyl-CoA pool | | | |
| | Addition of cerulenin | <i>E. coli</i> | [146, 170, 173, 178] |
| | | <i>C. glutamicum</i> | [158] |
| | O/E of ACC | <i>E. coli</i> | [108, 166, 172] |
| | | <i>S. cerevisiae</i> | [144] |
| | O/E of acetyl-CoA synthase | <i>E. coli</i> | [108, 166] |
| | K/O of acetate kinase | <i>E. coli</i> | [108, 166] |
| | O/E of <i>fabF</i> and <i>fabE</i> | <i>E. coli</i> | [166, 170] |
| | Expression of MatB and MatC from <i>Rhizobium trifolii</i> | <i>E. coli</i> | [168, 169] |
| | Repression of <i>fabD</i> | <i>E. coli</i> | [171] |
| | Up-regulation of glycolysis and down-regulation of the TCA cycle | <i>E. coli</i> | [165, 172, 173] |
| | | | |
| UDP-glucose availability | | | |
| | O/E of <i>pgm</i> , <i>galU</i> , <i>ndk</i> | <i>E. coli</i> | [169] |
| | K/O of <i>galE</i> and <i>galT</i> | <i>E. coli</i> | [169] |
| | O/E of <i>ycjU</i> | <i>E. coli</i> | [118] |
| | | | |
| NADPH availability | | | |
| | Deletion of <i>pgi</i> , <i>ppc</i> , and <i>pldA</i> | <i>E. coli</i> | [174] |
| | | | |
| Aromatic amino acid availability | | | |
| | | | |

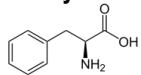
| | | | |
|--|--|----------------------|------------|
| | Modifications of the shikimate | <i>E. coli</i> | [168] |
| | pathway | <i>S. cerevisiae</i> | [177, 179] |
| | Reducing flux through the Ehrlich pathway | <i>S. cerevisiae</i> | [177] |

1033 O/E – overexpression, K/O – knock-out, TCA cycle – tricarboxylic acid cycle

1034

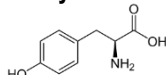
Amino acid metabolism

L-Phenylalanine



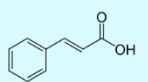
PAL

L-Tyrosine



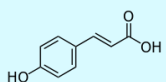
TAL

Cinnamic acid



C4H

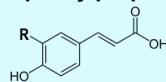
p-Coumaric acid



C3H

OMT

Other phenylpropanoids

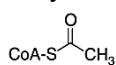


e.g. Caffeic acid

PHENOLIC ACIDS & Derivatives

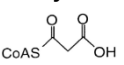
Sugar metabolism

Acetyl-CoA



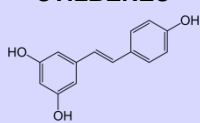
ACC

Malonyl-CoA



STS

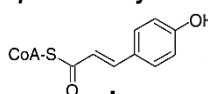
STILBENES



e.g. Resveratrol

4CL

p-Coumaroyl-CoA



LIGNIN
LIGNANS

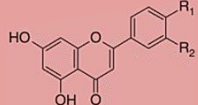
CHS

Chalcone

CHI

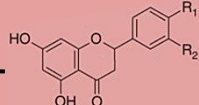
FLAVONOIDS

Flavones



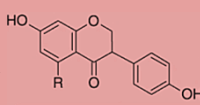
e.g. Apigenin

Flavanone



e.g. Naringenin

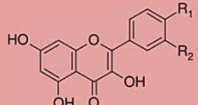
Isoflavones



e.g. Genistein

Isoflavonoids

Flavonols



e.g. Quercetin

FNS

IFS

F3H

FLS

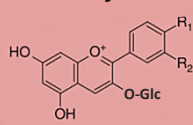
Dihydroflavonol

DFR

Leucoanthocyanidins
(Flavan-3,4-diols)

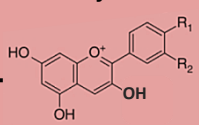
ANS

Anthocyanins



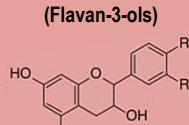
e.g. Chrysanthemin

Anthocyanidins



e.g. Pelargonidin

Catechins
(Flavan-3-ols)



e.g. (+)-catechin

Proanthocyanidins
(Condensed tannins)

3GT

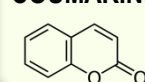
AAT

AMT

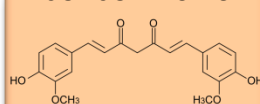
ANR

PHENYLPROPENES

COUMARINS



CURCUMINOIDS



e.g. Curcumin

AURONES

CUS

Figure 1. Plant polyphenols and their biosynthetic routes. Names of enzymes: 3GT, anthocyanidin 3-*O*-glycosyltransferase; 4CL, 4-coumaroyl-CoA ligase; AAT, anthocyanin acyltransferase; AMT, anthocyanin methyltransferase; ANR, anthocyanidin reductase; ANS, anthocyanidin synthase (leucoanthocyanidin dioxygenase); C3H, *p*-coumarate 3-hydroxylase; C4H, cinnamate 4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; CUS, curcuminoid synthase; DFR, dihydroflavonol 4-reductase; F3H, flavanone 3-hydroxylase; FLS, flavonol synthase; FNS, flavone synthase; IFS, isoflavone synthase; LAR, leucoanthocyanidin reductase; OMT, 3-*O*-methyltransferase; PAL, phenylalanine ammonia-lyase; STS, stilbene synthase; TAL, tyrosine ammonia-lyase ([1–3]).

- [1] Falcone Ferreyra ML, Rius SP, Casati P. Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Front Plant Sci* 2012; 3: 222.
- [2] Katsuyama Y, Kita T, Funa N, et al. Curcuminoid biosynthesis by two type III polyketide synthases in the herb *Curcuma longa*. *J Biol Chem* 2009; 284: 11160–70.
- [3] Vogt T. Phenylpropanoid Biosynthesis. *Mol Plant* 2010; 3: 2–20.